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THE APPARENT INHERITANCE OF AN ACQUIRED CHARACTER AND ITS EXPLANATION

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THE experiments described in the following pages grew out of an observation of one of the effects following injury to the eyes of *Drosophila* by heat. Several hours later the Malpighian tubules were found to have become deep red. The color remained for several weeks, suggesting that injury in one organ had brought about a more or less permanent change in another organ. The change seemed to be something more specific than a process of elimination of pigment (or a pigment-forming substance) from the eye through the excretory tubes. This effect on the tubules would not in itself seem very significant, but amongst the several thousand offspring from these flies with red Malpighian tubules a few offspring with red tubules were found in one culture. Since flies of this kind had never been observed before, it might have been concluded that an acquired character had been inherited. Evidence no more cogent than this had not infrequently been accepted as supporting the theory of transmission of an acquired character. But as the sequel will show, the explanation of this unusual occurrence has been found in an entirely different direction. (See page 104.)

From another point of view several other questions are to be considered in connection with an experiment of

this kind. For instance, does severe injury to the body, incidental to injury to the eyes, cause an increase in the number of mutations in the offspring, or in the descendants of the injured flies? If this should occur, would the mutations be more likely to appear in the organs that had been directly affected, or as likely to occur in any other part of the body? If mutational changes are, in general, more often visible (*i.e.*, detected) in the eyes than elsewhere, because slight differences in color are more noticeable than slight differences in other organs, and if more eye mutants are found in the descendants of the injured flies, the apparent correlation, if found, might still not be due to the injury to the eye, but rather to the greater frequency of recorded eye mutations. These and other questions could be answered only by an appeal to the evidence which, if on a sufficiently large scale, would serve to throw light on some of the theories that have been discussed for many years by advocates of one or another doctrine of evolution. Although more than fifty thousand flies were involved, some of these questions still remain in doubt, but others seem more definitely answered.

THE IMMEDIATE EFFECTS OF THE OPERATION

In the first experiments newly emerged adult females were used. It was found by trial that young individuals recover from the operation more often than the older ones. Their eyes are softer and more easily injured by the hot needle. A fly was slightly etherized. The end of a rather thick needle sharpened to a fine point was heated to red heat in a burner and immediately applied for a moment to the middle of the eye. A circular area of considerable extent changed color at once. A slight, quick touch is all that is necessary; a longer application kills the fly. The other eye was then treated in the same way. If the fly is quite young the surface may become indented, and may remain in this condition, or may later round out again. The flies are then laid on dry pieces

of paper, and put into a vial in which the air is slightly moist. If the flies are transferred to a food bottle at once they generally become stuck to the food before or soon after recovery. After several hours the flies are placed in a food bottle that has become partly dried out. Males are added three or four days later.

The injury to the eye is so severe that many of the flies do not recover; others that do recover are sluggish in their movements for several or many days and may not lay eggs for some time, and even then in reduced numbers. With practice the operation may be performed in such a way that most of the flies recover, but they are very weak at first, and, as stated, unless carefully handled become stuck sooner or later to the glass or food. Many are slow in taking food, and some of them never do so, possibly because the esophagus has been injured by the heat.

THE EFFECTS ON THE MALPIGHIAN TUBULES

After burning the eyes the tubules become colored in 20 to 30 minutes, and more conspicuously so in the course of a few hours. Under the microscope the color is seen to be contained in the granules of the cell wall. Yellow granules are normally present there, and the red color appears to be attached to or taken up by them. The same or similar granules are present in the lumen of the tubules, and pass into the lower part of the digestive tract, which later comes also to contain red granules if such are present in the tubes. In addition small patches of red may appear more anteriorly in the wall of the digestive tract. Probably there are cells or groups of cells in such localities that take up the color directly as do the cells of the Malpighian tubules. It seems likely that the eye-color in solution, or some precursor of the color, enters the blood or other fluids of the body and is taken over by the granules in the Malpighian tubules. It is practically certain that the red granules in the cells in the eyes are not carried as such to the tubules, first, because no such granules are visible in the blood, and, sec-

ond, because the yellow granules in the tubules appear themselves to be stained, and are not the red pigment granules of the eye.

If the eyes are punched with a *cold* needle, and the interior parts disturbed or destroyed, the tubules become only slightly colored and then only after some hours. The difference in effect may safely be ascribed to the greater injury caused by the hot needle; for, if the heads are cut off and the red eyes are crushed in Ringer's solution, and the pulp injected into the body cavity of another normal fly, the Malpighian tubules become colored in less than five minutes. Evidently heat is not necessary to produce the substance absorbed by the tubules. Most of the injections described below were made for me by Dr. Douglas Whitaker, who assisted in this part of the work.

When crushed material of red-eyed flies (in Ringer's solution) is injected into white-eyed flies, the tubules become red. Vermilion-eyed flies appear to have more yellow color in the tubules than have red-eyed flies. When the eyes of such flies are burnt, the tubules become very red. The eyes of maroon sepia are very dark. When the eyes are burnt, small yellow granules, sometimes slightly greenish, appear later in the tubules. Vermilion maroon sepia (with dark eyes) after burning develop the same color, brilliant yellow, in the tubules, as in the last case. The eosin eye color is pale orange yellow. After burning the eye, the tubules become pale yellow. Some brown or even red material appears in the duct. These few observations go to show, at least, that there is some relation between the color of the eyes and the color that appears in the tubules after burning.

The sheath of the testes in wild type flies is yellow. It is somewhat different in intensity of color in certain mutants, and in white-eyed flies there is almost no color in the sheath. When the red eye of the wild type is burnt the testes become deeper yellow or orange in color. The testes of vermilion-eyed flies are slightly more yel-

low than the testes of red-eyed flies, and when the eyes of vermilion flies have been burnt the testes become deeper yellow. When white-eyed flies are injected with pigment from red-eyed flies the tubules become red, as noted above, but no change in the color of the testes was recorded after five hours. It seems that the yellow color of the testes is related in some way to that of the eyes, and that after burning the color is enhanced, at least in red- and perhaps in vermilion-eyed flies.

THE BREEDING EXPERIMENTS

The First Experiment. The first experiment was made in February, 1928, with the wild Oregon stock. Several females with burnt eyes were put into the same bottle, but the offspring were often the product of only one or two of these. Fourteen bottles containing the females, mated after two or three days to normal males, produced 3,200 flies. Samples of these (F_1) were mated in pairs and gave, from 24 bottles, 1,830 flies. Again pairs (F_2) were made up from them, and 21 bottles gave 3,996 flies. In all there were 9,016 flies that came from the original females. Each fly was carefully examined, first for eye-color, second for the bristles on the head and thorax and third for wing variations. The flies were arranged in long rows on a white glass plate under a binocular, and first the eyes examined from the side. The flies were then turned over and the bristles examined, and finally the wings. Unless attention is drawn to one character at a time it is easy to overlook small differences. On the other hand, large or startling differences, such as body color or change in the shape of wings or legs, etc., are generally picked up at first sight.

Amongst these flies a few were found having one or another particular modification. These are generally defects or malformations, but there is no certain way of distinguishing, at sight, defectives from mutations, except by breeding tests. These were made in most cases. Of course long experience with *Drosophila* has familiarized

us with certain malformations, such as split thorax or torn wings or irregular abdominal rings, that appear not infrequently. Repeated tests have shown that these are not inherited. Nevertheless, it is unsafe to put too much reliance on this information, since mutations so similar in character as to be indistinguishable from such defects may occur. Owing to these conditions, and to others mentioned below, it is by no means safe to state too positively that only a given percentage of mutations occur in experiments carried out to get the mutation rates under experimental conditions.

The records of malformations and mutants in these experiments were as follows: In the first generation one wing was held out in one individual, and a Minute male (mutant type) was present in the same culture. In another culture (B) a male had one wing held out, and a narrow-eyed female was present. The male with one wing out bred to normal color gave all normal offspring. In a third culture the red eyes were slightly darker in one male (old?).

In the next generation from the last (B) the narrow-eyed female bred to a normal F_1 brother gave

N ♀	N ♂	Minute ♀	Minute ♂	Narrow ♀
111	164	26	1	2

Subsequently a stock of narrow was made that continued to produce normals and a few narrow. Thus one culture of 8 narrow females and 1 narrow male gave

N ♀	N ♂	Narrow ♀	Narrow ♂
32	36	2	1

Again, 5 normal females by 8 normal males out of narrow gave

N ♀	N ♂	Narrow ♀	Narrow ♂
109	93	8	1

This stock has been kept for two years. Bred in mass culture the narrow eye seldom appears, but bred in pairs some cultures give a few narrow and the latter if selected

produce more narrow. The simplest explanation is that narrow is a mutant type whose realization depends on a number of contributory genetic factors and environmental ones also.

In the F_3 from the narrow stock a male appeared with one wing held out. Also a Minute female (out of narrow to narrow) gave 164 normals only, but since Minute only appears at the end of culture their absence here was probably due to failure to run the culture to an end.

In another (F_1) culture (A) 27 normals were produced, and 5 males with twisted penis—the latter a recognized mutant type.

In culture (K) some F_1 flies (21 ♀ + 18 ♂) gave 427 normals and 5 yellow males. From the latter by suitable matings a yellow stock was made. Yellow is a mutant type that has been found several times in cultures of *Drosophila*. The other mutant types appeared in the later cultures of this line, namely, Minute and twisted penis.

The most remarkable finding here was a few males and females with slightly red Malpighian tubes—twenty-three individuals in all. When inbred on a large scale only normals developed indicating that the character was not inherited. Nevertheless, this occurrence called for further consideration, and led to the experiments described in the following section.

From culture (D) an F_1 male with wings out was mated to three normal sibs. Only normal offspring, F_2 , were produced. Two cultures of these F_2 gave in F_3 425 normals, and a very few flies whose wings were partly held out, but their identification in this respect is doubtful.

These results show that several mutant types appeared, one involving eye shape (narrow eye), one a body color (yellow) and another the bristles (Minute). One of these only is a new unknown mutant (narrow); the others are recognized as regular mutants, one yellow, the other Minute, which appears not infrequently in our stocks.

In addition there were a few other doubtful cases, either monstrosities or defects (not inherited), or multiple factor cases. Wings-out does not appear to be inherited, but twisted penis may be—at least certain kinds. There are indications that some of these characters are latent in the stocks bred. In any case it was observed that the injury to the eyes does not produce a preponderance of eye mutants.

EXPERIMENTAL PRODUCTION OF RED TUBULES

Several years ago I had observed some larvae of *Drosophila* feeding on the eyes of dead flies in a culture. The pigment taken into the digestive tract colored the intestine. It occurred to me, after finding the exceptional cases reported above, that if the red pigment were carried through the pupa stage it might appear in the Malpighian tubules of the imago.

The red eyes of 40 flies were crushed in a drop of tap water to which was added some growing yeast from a culture bottle. Larvae (Oregon stock) of various sizes were placed on this food. The digestive tract soon became filled with red food, and later the Malpighian tubules became deep red. The red tubules could be clearly seen through the walls of the larvae. When the flies that emerged (12 ♀, 8 ♂) were opened, the Malpighian tubules were deep red. One male only was not affected. Six other flies of this culture were kept alive and bred. After ten days the red tubules could still be seen through the body walls. The condition of the offspring will be recorded below.

In a second experiment 20 larva, of various sizes, were fed on the eyes of 40 flies plus yeast and agar. The next day the digestive tract had red food in it, and one larva observed under the microscope had red tubules. Four days later two had pupated (between 24 and 36 hours old). The Malpighian tubules were present and deeply colored. The remaining larvae were fed again on fresh food (eye-pigment and banana). Another pupa (be-

tween 1 and 2 hours old) had red tubules. The next day the four remaining pupae were opened. All had red tubules.

The results show that, when the red pigment is taken into the digestive tract of the larva, some constituent of the pigment passes through and stains the granules in the Malpighian tubules. These tubules remain intact through the pupa stages and are carried over into the imago, still retaining their color. The colored tubules can be seen through the body wall of larvae, pupae and adult.

On three occasions adult flies were fed on crushed eyes and yeast or banana. In two lots the digestive tract became filled with red food, but no color was detected in the tubules. In the third case several newly hatched Oregon stock flies were fed three times on crushed eyes plus banana. On the third day 20 flies were opened. In all 13 females there was red material in the digestive tract, and the tubules were faintly colored red or pinkish-yellow. In 4 males, with red in the intestine, the tubules showed a faint pinkish-yellow color; in 3 males there was no color detected. The difference in the sexes was marked, and may in part have been due to the greater amount of food taken by the females. The result shows that the coloring matter may pass through the digestive tract of the adult, but produces only a slight effect on the tubules. In fact, so faint was the color in some of the males that it might easily have been overlooked. It may safely be concluded that the red tubules in the original, exceptional offspring from burnt-eye flies were derived from food taken by the larvae rather than from food taken by the adult flies.

In order to examine the effect of other eye pigments than the red one, twelve Oregon larvae were fed on the crushed eyes (plus banana) of vermilion-eyed flies. The tubules became only faintly colored, pinkish-yellow. When twelve larvae from the vermilion stock were fed on vermilion pigment only a faint tinge of color could

be detected. On repetition, four larvae with much pigment material in the digestive tract again showed only a faint yellow-pink color in the tubules, but more pronounced than in the last case. The difference between the larvae from red-eyed and from vermilion-eyed stock in response to vermilion pigment fed to the larvae seemed a real but slight difference. When larvae from Oregon stock were fed on eosin eye pigment no certain effect on the tubules could be detected, although the effect may be too slight to give a positive result.

The fact that the Malpighian tubules become red when the larvae feed on the crushed eyes of dead flies leaves little doubt but that the exceptional flies recorded in the experiment arose in this way, especially when it is recalled that dead flies are frequently found on the surface of the food and the larvae have been seen eating out the contents of the eyes. The non-transmission of the induced character was shown by rearing offspring from flies with induced red tubules. A pair of such flies, from larvae that had fed on red eye pigment, produced 76 offspring (flies) that were opened and their Malpighian tubules examined. None showed any red color. Subsequently, 94 other flies (sibs) were looked over under a binocular but not dissected. None showed red tubules. The old flies were transferred to another bottle, and gave 137 flies, which, again, showed no red tubules. In another culture one female and four males with red tubules (from larvae fed on eye pigment) produced 82 offspring, which when opened showed no color in the Malpighian tubules. In addition 119 later offspring, examined with a binocular, showed no red. The old flies in a new bottle produced 87 flies without red in tubules. There were in all 595 offspring from these flies and none gave any evidence of the transmission of the induced character. These results are in line with those of the exceptional flies with red tubules in the second experiment that did not transmit the character.

Second Experiment. This experiment on flies from the same Oregon stock was carried out in June and July, 1928. Only the immediate (F_1) offspring of burnt females were examined, hence only recessive mutations in the X-chromosome would be revealed by the males, and dominants in any chromosome. In all 10,647 flies were examined, approximately half of which were males. Single lethal factors, if the result of the burning, would not be expected to appear, but if the female herself carried a lethal in half of her X-chromosomes its presence would be revealed by the sex ratio. No such ratios were found. The malformation and mutants that appeared were the following: In ten cultures, a few flies, whose wings slightly drooped, appeared, one in each culture. From none of these when tested were such flies found in the next generation. One female lacking bristles was recorded. She produced by a brother 238 normals. In three cultures males with a double penis appeared, four males in one culture, and one in each of the other two. Since these could not be bred they could not be tested further. The scattered occurrence of this character suggests that such a mutant type was present in the original stock. A female whose thorax was depressed anteriorly appeared. It gave only normal offspring. Split thorax is a defect that is familiar to students of *Drosophila*. Many tests have shown that it does not breed true. It is more frequent in certain stocks (vestigial) than in others. It appeared in two stocks; one of them bred to a normal brother gave 99 normal offspring. One fly (φ) had an indented eye with a rough surface in spots. Bred to a brother it produced 252 normals and one with triangular eyes. One fly had crumpled wings; it gave 50 normals in F_1 . One had lifted wings; it gave only normal F_1 and $F_2 \varphi$. One had both wings half unfolded; one held its wings out. The latter gave 85 normals in F_1 . One male with small bristles appeared. The F_1 and F_2 were all normals. While these tests were not always completely sufficient, they suffice to show whether the

character in question was a dominant autosomal character and in some cases whether, if in the male, it was sex-linked. Only in cases where the realization of the character, if due to a change of gene, is a multiple factor case, or due to exceptional environmental conditions, are the tests inadequate.

As a control ten pairs were made up from the Oregon stock, which produced 179, 149, 223, 235, 258, 121, 122, 68, 128 and 68 normal offspring. From each of these, nine pairs were made up (except in three cases when not all the cultures developed). In all they produced 24,047 flies. Amongst these one bottle (B-c) gave a Curly male, which bred to Curly female from stock gave 91 Curly and 48 normals, proving that the mutant (?) was Curly (a dominant lethal). In another culture (I-b) 94 normals and 123 Curlys were produced. When inbred they gave Curlys and normals. Unless contamination occurred the appearance of Curly in two cultures was due to mutation, but the chances are that contamination was present. One female appeared with folded wings. Only normal offspring were produced. One male had its wings held out. Bred to Oregon females it produced 30 normals. A mass culture of his sibs gave 87 normals.

As has been stated, the burnt females are very sluggish for several days and many of them die either as a result of the injury or because they can not feed. The surviving females do not begin to lay eggs for several days. The delay might in itself be supposed to cause abnormalities or even mutations quite aside from the injury. Therefore, a sort of control experiment was made with normal females that were kept without males for four days (although they were not starved). Fifteen pair cultures gave 2,458 offspring. Amongst these one fly held one wing out which was half as long as normal and rounded; one fly had a notch in its thorax and one wing stringy; one had drooping wings. There was also one Minute male. These defects (except the last) were simi-

lar to those obtained from the burnt flies. They were not further tested.

Third Experiment. This experiment was so planned that it would show whether the effect of the operation upon the X-chromosomes is specific (in the sense of being transmitted—as eye or tubule color modification), and also whether, in the offspring of injured flies, the frequency of lethals is greater than in the control. The method used by Muller for the detection of sex-linked lethals was followed. Females that had three sex-linked characters, echinus, cut¹, garnet², had their eyes burnt. They were mated to males of the yellow cross-veinless vermilion forked stock. The X-chromosome of the burnt female had three mutant genes and that of the male four mutant genes. The F₁ female had these two X-chromosomes. She was mated to what is called an X-ple male—i.e., one whose X-chromosome contains the seven recessive genes—scute, echinus, cross-veinless, cut⁶, vermilion, garnet², forked. Now, if a lethal X-chromosome gene were present in the germ-cells of the F₁ female, half the sons would die and a 2:1 sex ratio would appear. Such a ratio would occur if either of the X-chromosomes of the F₁ female carried a lethal. Which X-chromosome is the affected one could be determined by the character of the surviving males. If, for instance, the lethal is present in the ec ct⁶g² chromosomes this class of F₂ males will be the absent one, and conversely for the y cv v f characters. In cases where there might be some doubt of the sex ratio—and such cases are by no means as infrequent as may seem from what has been written—the absence of one of the two major classes of males furnished the proof that the ratio in question is one due to a sex-linked lethal and not a chance run, or one due to some other factor.

From one F₁ female (AA) 29 pairs gave no lethals; from another (BB) 42 pairs gave no lethals; from a third (DD) 47 pairs gave no lethals, although one case may have been such, but the full count of males was not made;

Fourth Experiment. In this experiment the eyes of the males were burnt. Despite the blindness that must have resulted, these males paired later with normal females, which is not surprising, since *Drosophila* males are known to mate with females in the dark. The procedure for the quick detection of lethals was one utilized by Muller. The injured males were mated to Bar females containing the Cl B chromosome. The C-factor prevents crossing over almost completely between the X-chromosomes. The other factor (1) is a recessive lethal that kills the Bar sons. The F_1 Bar daughters were mated to Oregon males in pairs, and the offspring examined for males. If no males are present, a new lethal

from the burnt male must have appeared. If a 2 to 1 ratio is found no such lethal has appeared. From one F_1 daughter 154 pairs were made up. No new lethals were discovered in any of the cultures.

At the time when the above matings were made a control was carried out in which normal flies of the same two stocks were combined in the same way. There were 123 pairs that gave no lethals.

Fifth Experiment. The eyes of three white females were burnt. They were bred to Oregon red-eyed males and gave 63 red-eyed daughters and 87 white-eyed sons—all normal. From a second culture containing the two surviving white females with burnt eyes, there were 377 normal offspring. Six cultures of F_1 gave 2218 red and white females and males—all normal except two. One of these was a red-eyed male with no wings. Bred to white sibs he gave red females and white males. Five of these F_1 females bred to two white males gave 136 offspring, all normal. It is not improbable here, since absence of wings was not transmitted, that the wing pads had been torn off at emergence of the fly. The other variation that appeared was a female with tilted wings that appeared in F_2 . Bred to F_2 sibs she produced 180 normal and 61 tilted-winged flies. From the latter, stock with such wings was reared—proving that a mutation had occurred.

Sixth Experiment. The unexpanded wing pads of newly hatched females were burnt. In one case two yellow females were burnt and mated to wild type males. The 347 offspring (187 ♀ + 160 yellow ♂) were normal. In another case the wing pads of an Oregon female were burnt. She was bred to male sibs. Twenty-three normal offspring were produced. From these, seven F_1 females and males gave 69 normals, and one F_1 female and two males gave 122 normal offspring.

DISCUSSION

In all there were about 24,567 flies examined, that came from burnt individuals, and about 28,456 in the control. In the experiments in which the flies were examined for

mutant characters after injury to the eyes, there were 19,663 females and males that might have shown dominants in any chromosome, and if half of them are reckoned as males there were about 9,000 that might have shown effects on either X-chromosome of the mother. The results show that there was no specific, inherited effect of injuring the eyes. It is more difficult to estimate whether the total number of proved mutant types was greater or less than "expected" in general, because there is not sufficient evidence to determine what this expectation should be. In the controls there were 28,000 flies and some of the same mutant types appeared here also. There is more than a suspicion that several of these types found in one or both cases came from the original Oregon stock. This stock has been kept running in mass culture for four years, but this is not a guarantee that it does not contain recessive mutant genes, for it is well known that in mass cultures the "weaker" mutants very seldom appear, although they may be continued under the surface by heterozygotes. It is true the controls came from pairs, but the number is not large enough to furnish accurate data as to the expectation of new mutant types appearing. It would, in fact, require very large numbers to even approximate to an accurate figure. Even then it is doubtful whether the estimate would suffice, because of the relatively larger number of defective individuals that appear. It is not easy to test all such cases since they grade down to minute changes that are unnoticeable. Even then, at least two generations are necessary to test them for recessive characters. The occurrence of multiple factor cases and those arising from uncontrollable environmental factors combined with the last create a difficulty that is in practice almost insuperable for reasonably accurate determination of expectation even in a form like *Drosophila*, where the large number of offspring per pair increases the probability of getting a fair sample of the germ plasm. In animals such as rabbits, guinea-pigs, rats and mice, where the output of an indi-

vidual is small, these difficulties are correspondingly enhanced.

In the other experiments in which tests were made for sex-linked recessive lethals, the number of cases is relatively small, but if the number of lethals that appear is as large as sometimes stated, and if these should be increased by the effect of injury and its subsequent influence on the whole animal, one might expect to find evidence of an effect. In fact, no lethal appeared in the offspring of the injured flies and only two in the control. It is probable that there are differences in different stocks in this respect, for the Oregon stock seems to contain or give rise to fewer sex-linked lethals than in some other cases reported by Muller, who has also reported differences in different stocks. These experiments, while undertaken primarily to show sex-linked lethals, also serve in part to show whether recessive characters in the male having a survival value result from the injury, and could in this sense be added to the other evidence had the individual flies been examined critically for such mutants, but this was not done.

Aside, then, from the possible effect of increasing the mutation rate by injury, for which the experiment may be said, I think, to furnish no striking evidence at best, the results give no evidence that severe injury to the eye brings about a preponderance of inherited effects (mutants) in that organ.

In the Carnegie Year Book, 1927-28, and 1929, references to these experiments and the course of their progress have been made. The question there raised was whether or not a higher percentage of mutation occurs in individuals whose eyes have been injured, either as eye-mutants or mutant characters in other parts of the body.

Owing to the malformations that occasionally appeared, it is difficult to determine accurately whether the results do or do not give a percentage of mutants higher than that shown by the same stock without treatment. At present, it can only be said that more mutants have been found in the offspring of the burnt flies than in the extensive control. These mutant characters appear in different parts of the body and not more frequently in the eyes than elsewhere.

This non-committal statement is no more than a statement of the facts there recorded. A somewhat more positive conclusion was drawn in the second communication (1928) in the light of further work where larger numbers were involved.

The results suffice to show, at least, that injuries to the eye do not produce offspring with defective eyes or any other modification in eye-color or structure, nor does the injury cause an increase in the occurrence of lethal genes in the X-chromosome. This conclusion holds, obviously, only within the limits of the numbers obtained.

One of the purposes of the experiment as originally planned was to determine whether the more general physiological effects of a serious operation gave an increase in the mutation rate comparable with that brought about by X-rays or radium. It seemed very improbable that the latter treatment did cause its effects in this way, since the direct effect of radium on chromosomes had been known for several years; nevertheless, it was, I thought, worth while to remove at least this possible criticism of the radium work. Muller has recently reported (*Scientific Monthly*, December, 1929), that he has examined the possible effectiveness of poisons and other influences than X-rays in producing lethals and other mutations on *Drosophila*, including a repetition of the experiment of injuring the eyes. His results were negative. In fact, Miss Mann had in 1923 made extensive experiments on the possible effect of injurious agents, arsenic, alcohol, quinine, morphine, methylene blue, lead acetate, lithium carbonate, copper sulphate, cold, in producing mutations, and in 1914 I had tried the effects of ether, alcohol, etc., with the same object in view. The results were again negative. It does not follow, of course, that other kinds of treatment may not be found that will give positive results either indirectly, by first affecting the general physiological condition of the body, or directly, by acting on the germ-cells.

THE DETERMINATION OF INTRA-CLASS AND INTER-CLASS EQUIVALENT PROBABILITY COEFFICIENTS OF CORRELATION

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I. INTRODUCTION

IN many cases the biometrician has to deal with the interrelationships between the three variables x , y and $w = x + y$, or with the three variables x , y and $v = x - y = y - x$. The determination of the possible product moment correlations is easy, either by the conventional product moment method or by the classical sum or difference methods, for which special routines have been indicated (3) (19). When the number of components of one of the variables, say $X = x_1 + x_2 + x_3 + \cdots + x_n$, is large, the determination of the coefficients by the use of moments about 0 as origin is also straightforward (11).

In interpreting the correlation between variables recorded in terms of $w = x + y$, a number of difficulties may arise. Certain of these have been cleared away by the suggestion of a coefficient measuring the relationship between w and the deviation of x or of y , from its probable value (4) and of the formula for the regression of the deviation of the dependent variable, x or y , from its probable value on the independent variable, w (12). This method has been applied in a number of investigations (which need not be cited here) to determine whether the proportionate contribution of x , or of y to the value of w is related to the magnitude of w . It has also been extended to the problem of the relationship between a variable and the deviation of an associated but not dependent variable from its probable value (14).

These methods leave untouched certain other problems of importance.

In many cases the biometrician has to deal with a variable, w , representing the total number of individuals falling in two alternative classes, x and y . For example, families of w children comprise x males and y females. Families of w children born during the period of fertility of the mother comprise x which survive to maturity and y which die in infancy or childhood. Plantings of w seeds may produce x seedlings per cultural unit area while y seeds may fail to develop. Of the w ovules formed by an ovary, x may produce embryos while y become abortive. Many other comparable relationships will suggest themselves. In such cases we may conveniently represent both the character and the frequency of the two alternative classes by x and y .

Now in such cases it may be of fundamental importance to determine whether individuals of character x (or y) are distributed at random within the values of w .

For example, the sex of offspring may be either male or female. In a family of size w , all the children may be male, all may be female or there may be any possible combination of individuals of the two sexes. A question of biological significance is whether there is a definite tendency for certain families to produce an excess of male offspring and for others to produce an excess of female offspring.¹ Again, in a house occupied by w individuals, a certain number, x , may become ill or may die during an epidemic. The problem as to whether infection introduced into a house by one individual will (under the conditions of sanitation and other precautions of isolation obtaining in the area) spread to other individuals of the same house is an important one. If there is such a spread of infection a material number of the houses should show a greater frequency of cases of morbidity or of mortality than is to be expected while others should show a smaller frequency of cases than is to be expected as a result of chance variations in the distribution of

¹ An investigation of this problem on human data is practically completed and will be published shortly.

cases. If instead of houses we write "families" or "fraternities" the possible influence of inheritance on the distribution of cases of morbidity must also be taken into account.

If, as we legitimately may, we tentatively regard the number w as a sample or class drawn from a population consisting of individuals of the characteristics x and y in the proportions $p = x/w$, $q = y/w$, we may properly inquire whether the observed variability of the number of individuals of character x represents merely the errors of random sampling which might be expected from the relative values of p and q , or whether it represents a distinct tendency for certain classes of w to be made up of disproportionately high frequencies of individuals of character x and for other classes to be made up of disproportionately high frequencies of individuals of character y . For example, Fisher (2, p. 69-73) used this method in considering the distribution of the numbers of the two sexes occurring in families of eight children. Again, Harris, Harrison and Wadley (21) have shown that the distribution of number of seedlings per hill in Sea-Island, Egyptian and upland cotton shows a large deviation from that given by a point-binomial. This evidences for the influence of extremely localized conditions on the germination of the seed or on the survival of the seedling up to the census date.

In dealing with all such problems by the methods so far indicated it is necessary to compare an empirical and a theoretical frequency distribution. The essential criterion of the differentiation is the probability of deviations between the two systems as large as or larger than those actually observed having arisen through random sampling. Obviously the requisite criterion is furnished by Karl Pearson's χ^2 and P test (29). This criterion is applicable to the frequencies of either continuous variables or of alternative categories, *e.g.*, Mendelian ratios as noted many years ago (7) and as more recently devel-

oped in greater detail by Fisher (2). It is ideal for practical application (beyond the determination of the theoretical frequency system) since it involves merely the calculation of $\chi^2 = [S((o - c)^2/c)]$, where o = observed frequency and c = calculated or theoretical frequency for any given frequency class, and S denotes summation for all classes. The value of P is to be determined from published tables (which extend the range of the small table given originally by Pearson) (29). These are provided by Elderton (1) and supplemented by tables calculated by Pearson (30). All are available in Pearson's "Tables for Statisticians and Biometricians" (31).

While Pearson's criterion represents one of the most fundamental advances in the field of biometric theory, certain limitations—both theoretical and practical—appear to be inherent in the above method *as applied to many biological investigations*.

(a) When w is small and is constant for a large number, N , of cases, it is easy to obtain the theoretical distribution $N(p + q)^w$. When, however, w is large, the calculation of the $w + 1$ terms of the binomial becomes laborious for a single value of w and becomes practically prohibitive if the number of values of w is large.

(b) It is impracticable in most cases to obviate the difficulties indicated under (a) by replacing the point binomial by a frequency curve. The normal or Laplace-Gaussian curve can be used in only those cases in which p and q do not differ widely, and the theoretical distribution is in consequence symmetrical. The condition $p = q$ will rarely be even approximately realized, and in a large majority of cases their values will depart materially from equality. Thus it would be necessary to select skew curves for the frequency distributions to avoid the calculation of point binomial distributions. Furthermore, whatever the relative magnitudes of p and q , these curves would have to have their range limited by o and w .

(c) In practically all cases, the number, N , of values of w, x, y , will be only moderately large. Certain sources of error which have recently been stressed in studies of limitations in the applicability of the contingency coefficient (26) (27) (28) then become effective. If w be large (or of smaller magnitude but highly variable), the number of theoretically possible classes of x will be very great. Thus for any value of w the point binomial will give $w + 1$ classes in which individuals of character x may fall. When N is small, certain of these theoretical frequency classes of x or y may be void. For such void cells $((o - c)^2/c) = (-c^2/c) = -c$. Thus the contribution of void cells will be the summed theoretical frequencies for such cells. If the number of void cells be large and N be substantial, the contribution of such void cells to χ^2 will be large. Furthermore, in the concentration of the actual frequencies into a very few of the $w + 1$ frequency classes (either through the errors of random sampling or through the influence of some unrecognized biological factor) the numerator of $(o - c)^2/c$ will be large as compared with the denominator.

Finally, as emphasized by Pearson, the fact that observational frequencies must be recorded in units whereas calculated frequencies have fractional values will also tend to render χ^2 unduly large when $w + 1$ is large and N is small.

In the case of a large value of w represented by but few observations all these factors will contribute to the amassing of large values of χ^2 . Thus, as Pearson clearly recognized nearly thirty years ago, the χ^2 and P test of the agreement of observation and theory becomes illusory when the number of classes becomes unduly large.

(d) Finally, the interpretation of the physical quantity χ^2 in terms of P presents two serious difficulties in the case of the kind of problem considered in this paper. In many instances the significant figures of P are found to lie far beyond the limits of $P = .000000$ of Elderton's

table (1). If the demonstration that (a) the observed system can not reasonably be supposed to have arisen from the theoretical distribution through random sampling, or, stating the matter in a different way, that (b) the graduation formula adopted is not suitable as a generalization from the sample in question to an infinite population, is all that is required, the range $P=1.000000$ to $P=0.000000$ is ample, since chances of 999,999 to 1 should satisfy any reasonable worker.

For certain purposes, however, we require much more than this. We need to be able to compare the probabilities (or improbabilities) of the agreement between observation and theory in two or more series of data. In many cases these values must be written in terms of P which are so small that the results are altogether incomprehensible. We must, therefore, seek some other method of expressing the deviation of the results of observation from probability.

II. DEVELOPMENT OF METHODS

Remembering that any one of the w individuals may be of the character x or y it is practicable to make all possible permutations of the individuals of any class of w in the 2×2 -fold distribution

	x	y
x	n_{xx}	n_{xy}
y	n_{yx}	n_{yy}

Thus if there be four individuals all of character x , $n_{xx}=12$, and all other cells of the fourfold distribution are void. If there be three individuals of character x and one of character y , the combinations are $n_{xx}=6$, $n_{xy}=3$, $n_{yx}=3$, $n_{yy}=0$.

Summing the combination frequencies for all N values of w , we obtain a table of association within the classes of w to which the individuals of characters x and y belong.

Now such tables are nothing more than intra-class (8) contingency surfaces representing the permutation frequencies of the two alternative characters of the individuals of a class. The data are, therefore, presented in a form amenable to treatment by Pearson's equivalent probability correlation method (30). After the conceptions of the present paper were formulated, this method was applied to the special problem of the correlation between the fates of the seeds planted in the same hill (22).

The purpose of the present paper is (a) to indicate the facility with which the necessary 2x2-fold tables required for the determination of intra-class and inter-class equivalent probability correlation coefficients may be formed from the frequency distributions of the variables by utilizing the method of moments about 0 as origin frequently employed in determining variation constants and correlations (5) (15), and (b) to illustrate in small part the range of applicability of the method.

Three classes of cases (A-C) require consideration.

(A) In general the frequency distribution of x , y , $x + y = w$, $x - y = v$, and possibly the correlation surfaces showing the relationship between w and x , w and y or x and y will be available.

The frequencies of the 2x2-fold table

	x	y	Totals
x	n_{xx}	n_{xy}	$n_{xx} + n_{xy}$
y	n_{yx}	n_{yy}	$n_{yx} + n_{yy}$
	$n_{xx} + n_{yx}$	$n_{xy} + n_{yy}$	$S[w(w-1)]$

may be written at once in terms of the first and second moments and product moments of the variables. Thus

$$n_{xx} = S(x^2) - S(x) = S(w^2) - S(y^2) - 2S(xy) - S(x) \\ = 2S(xy) - S(y^2) + S(v^2) - S(x) \quad \text{.....(i)}$$

$$n_{yy} = S(y^2) - S(y) = S(w^2) - S(x^2) - 2S(xy) - S(y) \\ = 2S(xy) - S(x^2) + S(v^2) - S(y) \quad \text{.....(ii)}$$

Since by definition the intra-class correlation or contingency surface here employed is symmetrical

$$n_{xy} = n_{yx} = S(xy) = \frac{1}{2}[S(w^2) - S(x^2) - S(y^2)] \\ = \frac{1}{2}[S(x^2) + S(y^2) - S(v^2)] \\ = S(wx) - S(x^2) \\ = S(wy) - S(y^2) \quad \text{.....(iii)}$$

Remembering that (19) if S denotes summation of the summations of arrays of a correlation surface or of the N individuals of the sample as may be indicated by the context,

$$S(x) = S[\Sigma(x_y)], \quad S(x^2) = S[\Sigma(x_y^2)], \\ S(y) = S[\Sigma(y_x)], \quad S(y^2) = S[\Sigma(y_x^2)], \\ S(x+y) = S(x) + S(y) = S[\Sigma(x_y)] + S[\Sigma(y_x)], \\ S(x-y) = S(x) - S(y) = S[\Sigma(x_y)] - S[\Sigma(y_x)], \\ S[(x-y)^2] = S(x^2) + S(y^2) - 2S(xy) = S[\Sigma(y_x^2)] + S(n_x x^2) \\ - 2S[x \Sigma(y_x)] = S[\Sigma(x_y^2)] + S(n_y y^2) - 2S[y \Sigma(x_y)],$$

while for $S(x+y)^2$ the equations are the same except that the final term is positive, it is clear that the fourfold table is readily obtainable from the moments of any two of the variables.

While the foregoing equations are ideal when the frequency distributions or the correlation surfaces of two or more of the variables are given, it may sometimes prove necessary to form the equivalent probability contingency tables from the means, standard deviations and correlations. Let \bar{x} , \bar{y} , \bar{w} , \bar{v} be the means, σ_x , σ_y , σ_w , σ_v the standard deviations, r_{xy} , r_{wx} , r_{wy} , etc., the correlations. By well-known algebraic transformations (i), (ii), (iii) become respectively (iv), (v) and (vi),

$$n_{xx} = N[\sigma_x^2 + \bar{x}(\bar{x} - 1)] \quad \text{.....(iv)}$$

$$n_{yy} = N[\sigma_y^2 + \bar{y}(\bar{y} - 1)] \quad \text{.....(v)}$$

$$n_{xy} = n_{yx} = S(xy) = \frac{1}{2}N[\sigma_x^2 + \bar{x}^2 + \sigma_y^2 + \bar{y}^2 - \sigma_v^2 - \bar{v}^2] \\ = N[r_{wx}\sigma_w\sigma_x + wx - \sigma_x^2 - x^2] = N[r_{xy}\sigma_x\sigma_y + \bar{x}\bar{y}] \quad \text{.....(vi)}$$

(B) Remembering that in the preceding discussion w represents the number of individuals in a class or sample, the individual members of which may possess either of the two alternative characters x or y , we now consider a special case which may sometimes arise.

In certain instances the individuals constituting any one of the m classes or samples may be logically subdivisible into subclasses, say $w_1, w_2, w_3, \dots, w_n$. In such cases it may be desirable to determine the correlations between the characters of associated individuals constituting the class for (a) the individuals which belong to the same subclass and for (b) the individuals of the class which belong to different subclasses. The correlation for (b) has been termed a fractional intra-class correlation (8). For convenience let us designate the combination or major classes as $W = \Sigma(w)$, where the Σ denotes the summation of the number of individuals, w , falling in each of the subclasses of W . The total sample will then be $S[\Sigma(w)] = S(w) = S(W) = N$, where S denotes summation of subclasses or of major classes throughout the sample as may be indicated by the context.

Now in case (a) the major classes are disregarded and a 2x2-fold table is formed by application of equations (i)-(iii), or (iv)-(vi). In case (b) the permutation frequencies within the minor classes must be deducted from the permutation frequencies within the major classes.

The permutation frequencies within the subclasses (w) may be designated as $n_{xx}, n_{yy}, n_{xy}, n_{yx}$ as heretofore. The permutation frequencies within the combinations or major classes (W) may be designated by $n_{XX}, n_{YY}, n_{XY}, n_{YX}$. Finally, the permutation frequencies for the individuals within the same class but within different subclasses of W may be designated as $n_{x'x'}, n_{y'y'}, n_{x'y'}, n_{y'x'}$.

The following relations then hold:

$$\begin{aligned} n_{x'x'} &= n_{XX} - n_{xx} = [S(X^2) - S(X)] - [S(x^2) - S(x)] \\ &= [S(W^2) - S(Y^2) - 2S(XY) - S(X)] - [S(w^2) - S(y^2) - 2S(xy) - S(x)] \\ &= [2S(XY) - S(Y^2) + S(Y^2) - S(X)] - [2S(xy) - S(y^2) + S(y^2) - S(x)] \dots (vii) \end{aligned}$$

$$\begin{aligned}
 n_{y'y'} &= n_{yy} = [S(Y^2) - S(Y)] - [S(y^2) - S(y)] \\
 &= [S(W^2) - S(X^2) - 2S(XY) - S(Y)] - [S(w^2) - S(x^2) - 2S(xy) - S(y)] \\
 &= [2S(XY) - S(X^2) + S(Y^2) - S(Y)] - [2S(xy) - S(x^2) + S(y^2) - S(y)] \dots (viii) \\
 n_{y'x'} &= n_{x'y'} = n_{yx} - n_{yx} = S(XY) - S(xy) \\
 &= \frac{1}{2}[S(W^2) - S(X^2) - S(Y^2)] - \frac{1}{2}[S(w^2) - S(x^2) - S(y^2)] \\
 &= \frac{1}{2}[S(X^2) + S(Y^2) - S(V^2)] - \frac{1}{2}[S(x^2) + S(y^2) - S(v^2)] \\
 &= [S(WX) - S(X^2)] - [S(wx) - S(x^2)] \\
 &= [S(WY) - S(Y^2)] - [S(wy) - S(y^2)] \dots (ix)
 \end{aligned}$$

These equations, like (i)-(iii), are ideal for practical work when frequency distributions or surfaces are available. When means, standard deviations and coefficients of correlation only are at hand, equations quite comparable with (iv)-(vi) may be written by noting that the relations for X, Y, W, V are identical with those for x, y, w, v .

(C) In some investigations it may be necessary to determine the equivalent probability inter-class correlation between associated classes, w_1 and w_2 . Denoting the values of x, y and v of the two classes by the subscript numerals 1 and 2, we may determine the equivalent probability inter-class contingency surface

	x_2	y_2	Totals
x_1	$n_{x_1x_2}$	$n_{x_1y_2}$	$n_{x_1x_2} + n_{x_1y_2}$
y_1	$n_{y_1x_2}$	$n_{y_1y_2}$	$n_{y_1x_2} + n_{y_1y_2}$
Totals	$n_{x_1x_2} + n_{y_1x_2}$	$n_{x_1y_2} + n_{y_1y_2}$	$S(w_1w_2)$

from the formulas

$$n_{x_1x_2} = S(x_1x_2) = S(w_1w_2) - S(x_1y_2) - S(y_1x_2) - S(y_1y_2) \dots (x)$$

$$n_{y_1y_2} = S(y_1y_2) = S(w_1w_2) - S(x_1x_2) - S(x_1y_2) - S(y_1x_2) \dots (xi)$$

$$n_{x_1y_2} = S(x_1y_2) = S(w_1w_2) - S(x_1x_2) - S(y_1x_2) - S(y_1y_2) \dots (xii)$$

$$n_{y_1x_2} = S(y_1x_2) = S(w_1w_2) - S(y_1y_2) - S(x_1y_2) - S(x_1x_2) \dots (xiii)$$

If the classes are all of uniform size formulas (xii) and (xiii) become

$$n_{x_1y_2} = S(x_1y_2) = n\sum(x_1) - S(x_1x_2) \dots\dots\dots (xiv)$$

$$n_{y_1x_2} = S(y_1x_2) = n\sum(x_2) - S(x_1x_2) \dots\dots\dots (xv)$$

Formulas (xii) and (xiii) assume that the contingency surface is unsymmetrical. In some instances it is desirable to use symmetrical surfaces. In such cases the values of $n_{x_1x_2}$, $n_{y_1y_2}$ are doubled and $n_{x_1y_2}$, $n_{y_1x_2}$ are each replaced by $n_{x_1y_2} + n_{y_1x_2}$. Under these conditions the total frequency of the surface becomes $2S(w_1w_2)$.

In terms of the statistical constants, equations (x)-(xiii) become respectively

$$\begin{aligned} n_{x_1x_2} &= S(x_1x_2) = N(r_{x_1x_2}\sigma_{x_1}\sigma_{x_2} + \bar{x}_1\bar{x}_2) \\ &= N[rw_1w_2\sigma_{w_1}\sigma_{w_2} + \bar{w}_1\bar{w}_2 - r_{x_1y_2}\sigma_{x_1}\sigma_{y_2} - \bar{x}_1\bar{y}_2 - r_{y_1x_2}\sigma_{y_1}\sigma_{x_2} \\ &\quad - \bar{y}_1\bar{x}_2 - r_{y_1y_2}\sigma_{y_1}\sigma_{y_2} - \bar{y}_1\bar{y}_2] \dots\dots\dots (xvi) \end{aligned}$$

$$\begin{aligned} n_{y_1y_2} &= S(y_1y_2) = N(r_{y_1y_2}\sigma_{y_1}\sigma_{y_2} + \bar{y}_1\bar{y}_2) \\ &= N[rw_1w_2\sigma_{w_1}\sigma_{w_2} + \bar{w}_1\bar{w}_2 - r_{x_1x_2}\sigma_{x_1}\sigma_{x_2} - \bar{x}_1\bar{x}_2 - r_{x_1y_2}\sigma_{x_1}\sigma_{y_2} \\ &\quad - \bar{x}_1\bar{y}_2 - r_{y_1x_2}\sigma_{y_1}\sigma_{x_2} - \bar{y}_1\bar{x}_2] \dots\dots\dots (xvii) \end{aligned}$$

$$\begin{aligned} n_{x_1y_2} &= S(x_1y_2) = N(r_{x_1y_2}\sigma_{x_1}\sigma_{y_2} + \bar{y}_1\bar{x}_2) \\ &= N[rw_1w_2\sigma_{w_1}\sigma_{w_2} + \bar{w}_1\bar{w}_2 - r_{x_1x_2}\sigma_{x_1}\sigma_{x_2} - \bar{x}_1\bar{x}_2 - r_{y_1y_2}\sigma_{y_1}\sigma_{y_2} \\ &\quad - \bar{y}_1\bar{y}_2 - r_{y_1x_2}\sigma_{y_1}\sigma_{x_2} - \bar{y}_1\bar{x}_2] \dots\dots\dots (xviii) \end{aligned}$$

$$\begin{aligned} n_{y_1x_2} &= S(y_1x_2) = N(r_{y_1x_2}\sigma_{x_2}\sigma_{y_1} + \bar{y}_1\bar{x}_2) \\ &= N[rw_1w_2\sigma_{w_1}\sigma_{w_2} + \bar{w}_1\bar{w}_2 - r_{y_1y_2}\sigma_{y_1}\sigma_{y_2} - \bar{y}_1\bar{y}_2 - r_{x_1x_2}\sigma_{x_1}\sigma_{x_2} \\ &\quad - \bar{x}_1\bar{x}_2 - r_{x_1y_2}\sigma_{x_1}\sigma_{y_2} - \bar{x}_1\bar{y}_2] \dots\dots\dots (xix) \end{aligned}$$

III. ILLUSTRATION OF APPLICABILITY OF METHOD

These methods are of very wide applicability. Since they will be employed in several investigations which are nearly completed we give a single illustration which involves the three cases discussed above. We consider the procedure necessary in determining the influence of highly localized conditions on seedling stand.

Ideally the problem should be investigated by the determination of the correlation between each of the possible environmental variables of the soil and the germination or establishment rate of the seeds planted. Such

methods of research are, however, in their first stages of development. That they may sometime be applied successfully to large groups of problems is indicated by very satisfactory results for the correlation between the electrical conductivity of the saturated soil mass and the physicochemical properties of the plant tissue fluids (16), and by less striking results for the correlation between the chloride and sulphate content of the soil and the plant tissue fluid properties (23), and between soil salinity and flowering date in cotton (20). As far as we are aware the only study of the relationship between a definitely measured property of the soil and the seedling stand produced is a single preliminary study of the correlation between soil salinity and seedling stand in cotton (18).

It will be a long time before we have sufficiently accurate and rapid methods of measuring the properties of the soil to make possible correlations between all the variables of the soil and the seedling stand produced. Pending the advances which must be made toward this desirable end, it is necessary to measure the relationship between the properties of the soil and seedling stand indirectly by determining the correlation between the fate of seeds planted in association over the experimental field (17).

This method of procedure has been successfully used in the measurement of substratum heterogeneity (9) and in the demonstration of the generality of this factor in the determination of crop yields (13). These results have been substantiated by the use of the method of inter-annual correlation (10) in the investigation of the permanence of differences in the plots of the experimental field (24) (25).

The investigation of the influence of localized conditions on seedling stand does not differ in principle from that employed in dealing with other plant characters, but only in the details of method imposed by the fact that if

we deal with single seeds they can be classed in only two alternative categories, survived and died.

If the number of seeds planted per hill be kept constant and if a number of hills be included in each plot, the number per hill of seeds germinating and surviving to a given date may be considered as a quantitatively measured variable, and intra-class or inter-class correlation coefficients determined by the usual procedure (8). If, however, the areas on which the seeds are planted be made so small that they can not be further subdivided, it becomes necessary to consider the relationship between the fate of the seeds in terms of the symmetrical four-fold distribution

	<i>s</i>	<i>d</i>	Total
<i>s</i>	n_{ss}	n_{sd}	$n_{ss} + n_{sd}$
<i>d</i>	n_{ds}	n_{dd}	$n_{ds} + n_{dd}$
Total	$n_{ss} + n_{ds}$	$n_{sd} + n_{dd}$	N

As will be shown later, it may be desirable to extend this method to wider areas to which the ordinary intra-class and inter-class theory might be applied.

The ease with which all these coefficients may be determined from the class moments may be illustrated by data for an experiment on the germination of Pima Egyptian and Acala upland cotton made at the U. S. Field Station at Sacaton, Arizona, in 1926. Two plots were planted with light irrigation and two plots with normal irrigation. Six seeds were regularly planted in each hill. The hills were arranged in successive subplots each of seven hills. Subplots of Pima Egyptian and Acala upland cotton were arranged alternately.

There were sixteen rows under each treatment, each row divided into nineteen sections of seven hills of Pima Egyptian alternating with seven hills of Acala upland cotton. Thus there were 304 such subplots representing a total of 2,128 hills of each variety under each of the two

experimental conditions. Our purpose is to determine the extent to which highly localized conditions in these small areas (each 52×400 feet) influence seedling stand.

All necessary data for the determinations of the correlations required may be summarized in two tables.

Table I gives the frequency distribution of the number of seedlings per hill in the 2,128 hills of each of the two

TABLE I
FREQUENCY DISTRIBUTION OF NUMBER OF SEEDLINGS PER HILL IN PIMA AND
ACALA COTTON (EXP. 1/26)

Number of seedlings per hill	Normal irrigation (Plots C3-16, C3-17)		Light irrigation (Plots C3-18, C3-19)	
	Pima	Acala	Pima	Acala
0	363	365	65	136
1	73	145	42	180
2	83	234	67	308
3	144	341	182	452
4	302	470	343	520
5	510	399	692	386
6	653	174	737	146
Total	2128	2128	2128	2128

varieties as grown under each of the two experimental conditions.

The first column of Table II gives the total number of seedlings produced per subplot (each of seven hills) in either Pima Egyptian or Acala upland. The second, fourth, sixth and eighth columns give the frequencies of these numbers of seedlings per subplot as they were actually found for Pima, P, and Acala, A, cotton. Immediately following these four columns, and bearing the captions $S[\Sigma(A)]$, $S[\Sigma(P)]$, are columns three, five, seven and nine, which give the total numbers of seedlings on the subplots of Pima cotton, $S[\Sigma(P)]$, associated with subplots of Acala cotton with the numbers of seedlings of Acala cotton indicated in the first column, or the total number of seedlings on the subplots of Acala cotton,

$S[\Sigma(A)]$, associated with the number of seedlings of Pima cotton indicated in the first column. Thus Table II comprises two condensed correlation tables (6), that

TABLE II

CONDENSED CORRELATION TABLES FOR RELATIONSHIPS BETWEEN TOTAL NUMBER OF SEEDLINGS PER SUBPLOT, EACH OF SEVEN HILLS, IN PIMA AND ACALA COTTON

Normal irrigation (Plots C3-16, C3-17)					Light irrigation (Plots C3-18, C3-19)			
Number of seedlings								
S	f_p	$S[\Sigma(A)]$	f_A	$S[\Sigma(P)]$	f_p	$S[\Sigma(A)]$	f_A	$S[\Sigma(P)]$
0	3	22	1	18	—	—	1	25
1	2	20	2	28	—	—	—	—
2	1	9	1	12	—	—	—	—
3	—	—	—	—	1	11	—	—
4	4	52	—	—	—	—	—	—
5	2	31	1	19	—	—	1	28
6	—	—	5	66	1	8	—	—
7	1	23	2	42	—	—	—	—
8	2	20	3	47	—	—	2	41
9	2	61	6	117	—	—	—	—
10	1	10	7	164	—	—	2	63
11	1	21	6	117	1	25	3	71
12	4	46	6	119	—	—	—	—
13	1	23	5	81	—	—	2	56
14	4	69	8	154	—	—	5	161
15	5	118	6	163	—	—	5	176
16	9	146	11	264	3	64	8	257
17	7	112	16	458	—	—	9	311
18	6	95	9	248	—	—	15	464
19	6	94	14	381	1	18	12	404
20	6	156	9	266	1	23	19	608
21	5	76	15	427	4	88	25	811
22	10	212	17	560	—	—	18	616
23	12	231	22	601	1	23	23	762
24	5	85	13	370	4	88	15	491
25	5	112	23	722	7	141	29	946
26	8	164	12	379	7	157	28	970
27	9	186	20	591	10	246	25	805
28	16	351	10	330	9	179	17	584
29	16	391	11	329	10	228	16	528
30	12	272	8	248	17	416	8	259
31	12	258	9	272	18	426	7	239
32	19	441	8	223	19	449	3	106
33	16	379	7	213	26	604	3	100
34	12	311	4	131	27	589	2	62
35	15	326	4	112	35	823	—	—
36	17	428	1	38	35	840	1	32
37	15	391	—	—	23	554	—	—
38	15	381	1	37	22	551	—	—
39	12	270	—	—	12	251	—	—
40	4	106	—	—	6	141	—	—
41	1	22	—	—	3	75	—	—
42	1	34	—	—	1	20	—	—

is, tables in which the first moments of the values of y associated with given values of x , or the first moments of x associated with given values of y are entered.

From such a table sample means and standard deviations, the product moments, and the means of arrays for the correlations between the number of seedlings of the two varieties in associated pairs of subplots may be readily determined by the methods of moments about 0 as origin (5) for the determination of the relationship between the number of seeds per plot germinating and surviving to a given date in associated plots of Pima Egyptian and Acala upland cotton. For the two experimental fields under different irrigation influences, we find

$$\begin{array}{llll} \text{For light irrigation,} & N=304, & S(A) = 7038, \\ S(A^2) = 171080, & \bar{A} = 23.1513, & \sigma_A = 5.1749, & S(P) = 9976, \\ S(P^2) = 335884, & \bar{P} = 32.8158, & \sigma_P = 5.2920, & S(AP) = 232381, \\ & & r_{AP} = +.1710 \pm .0375. \end{array}$$

$$\begin{array}{llll} \text{For normal irrigation,} & N=304, & S(A) = 6555, \\ S(A^2) = 158121, & \bar{A} = 21.5625, & \sigma_A = 7.4292, & S(P) = 8347, \\ S(P^2) = 255321, & \bar{P} = 27.4572, & \sigma_P = 9.2721, & S(AP) = 189125, \\ & & r_{AP} = +.4366 \pm .0313. \end{array}$$

For the determination of the intra-class correlation between the number of seedlings per hill in the same subplot we require merely the first and second moments of the number of seedlings per hill from the frequency distributions of Table I in addition to the second moments for number of seedlings per subplot derived from Table II and given above. The moments and constants for number of seedlings per hill are:

$$\begin{array}{llll} \text{For light irrigation,} & S(a) = 7038, & S(a^2) = 28706, \\ \bar{a} = 3.3073, & \sigma_a = 1.5973, & S(p) = 9976, & S(p^2) = 51268, \\ \bar{p} = 4.6880, & \sigma_p = 1.4543. \end{array}$$

$$\begin{array}{llll} \text{For normal irrigation,} & S(a) = 6555, & S(a^2) = 27909, \\ \bar{a} = 3.0804, & \sigma_a = 1.9043, & S(p) = 8347, & S(p^2) = 42791, \\ \bar{p} = 3.9225, & \sigma_p = 2.1732. \end{array}$$

Remembering that the weighted N is $S[n(n-1)] = mn(n-1) = 304 \times 7 \times 6 = 12768$, since the number n of

seedlings per subplot is constant for the m subplots, we have for Acala series,

$$\frac{1}{\sigma_a^2} \{ [S(A^2) - S(a^2)] / [mn(n-1)] - \bar{a}^2 \}.$$

This formula gives for the four series:

For Pima, normal irrigation, $r_{pp} = .2667 \pm .0136$.

“ “ light irrigation, $r_{pp} = .1485 \pm .0143$.

For Acala, normal irrigation, $r_{aa} = .1957 \pm .0141$.

“ “ light irrigation, $r_{aa} = .0833 \pm .0145$.

For the inter-class correlation between number of seedlings per hill in associated subplots of Pima and Acala cotton

$$r_{pa} = \frac{1}{\sigma_p \sigma_a} [S(PA) / mn^2 - \bar{p}\bar{a}].$$

Evaluation gives

For normal irrigation, $r_{pa} = .1483 \pm .0143$.

For light irrigation, $r_{pa} = .0411 \pm .0146$.

In all these cases the probable errors for the intra-class and inter-class correlations were calculated on the basis of the actual number of hills, $N = 2128$.

These results exhaust the possibilities of the product moment methods. They show that there is a low to medium correlation between the number of seedlings of the two varieties of cotton produced out of the forty-two possible on adjoining subplots. The coefficient is far higher for normal irrigation than for light irrigation. When the correlations are based on the number of seedlings produced per individual hill, as in the intra-class and inter-class correlation method, the coefficients are much lower than when based on the total numbers per subplot. This is exactly the result to be expected when the estimate of the seedling-producing capacity of the soil is based on single hills instead of on groups of seven hills. All the coefficients are, however, positive in sign, and may be considered significant in comparison with their probable errors. The differences noted between the light

and normal irrigation series are apparent in both methods of determining the correlations.

Turning now to the equivalent probability correlations, we note that these may measure (a) the intra-class correlation between fate of the seeds planted in the same hill; (b) the intra-class correlation between fate of the seeds planted in the same subplot; (c) the inter-class correlation between the fate of the seeds planted in two different subplots which are closely associated in space; (d) the correlation between the fate of the seeds planted in different hills of the same subplot.

In determining the correlation between the fate of seeds within a hill, w , the total number of seeds planted per hill, remains constant throughout; x will be the number of seeds germinating and surviving per hill and y the number failing to germinate or to survive. The frequencies for the fourfold tables may be computed from

TABLE III

INTRA-CLASS EQUIVALENT PROBABILITY CORRELATION, r_p , BETWEEN FATE OF SEEDS PLANTED IN THE SAME HILL

		n_{xx}	n_{xy}	n_{yx}	n_{yy}	χ^2	r_p
Normal irrigation (Plots C3-16, C3-17)							
Pima	o	34444.0	7291.0	7291.0	14814.0	15671.99	.8262
"	c	27284.0	14451.0	14451.0	7654.0		
"	o-c	+7160.0	-7160.0	-7160.0	+7160.0		
Acala	o	21354.0	11421.0	11421.0	19644.0	5144.91	.4447
"	c	16826.5	15948.5	15948.5	15116.5		
"	o-c	+4527.5	-4527.5	-4527.5	+4527.5		
Light irrigation (Plots C3-18, C3-19)							
Pima	o	41292.0	8588.0	8588.0	5372.0	2886.57	.4191
"	c	38972.6	10907.4	10907.4	3052.6		
"	o-c	+2319.4	-2319.4	-2319.4	+2319.4		
Acala	o	21668.0	13522.0	13522.0	15128.0	1319.58	.2252
"	c	19397.5	15792.5	15792.5	12857.5		
"	o-c	+2270.5	-2270.5	-2270.5	+2270.5		

formulas (i), (ii) and (iii), the moments for which may be obtained directly from Table I.

Table III shows the frequencies as thus computed for the two varieties of cotton under each of the two experimental conditions and the deviations of these frequencies from those calculated on the assumption that x and y are associated with each other in the same proportion in which they are found in the total population. It also shows the values of χ^2 measuring the deviations of the empirical from the theoretical frequencies. The positive nature of the correlation is shown at once by the positive signs of $o - c$ for like combinations of x and y , where $o =$ observed and $c =$ calculated frequencies. The values of χ^2 are given directly by $S\left[\frac{(o - c)^2}{c}\right]$ or may be calculated without the determination of the theoretical frequencies from Pearson's (30) formula which in our notation is

$$\chi^2 = \frac{(n_{x_1x_2}n_{y_1y_2} - n_{x_1y_2}n_{y_1x_2})^2 \cdot [S(w_1w_2)]}{(n_{x_1x_2} + n_{x_1y_2})(n_{y_1x_2} + n_{y_1y_2})(n_{x_1x_2} + n_{y_1x_2})(n_{x_1y_2} + n_{y_1y_2})}.$$

The values of r_p , the intra-class equivalent probability r , are derived in the usual manner by the use of Pearson's (30), (31) tables.

As an example of the routine we take the determination of the value of r_p for seeds of Acala in the same hill. The data from Table III are in Pearson's notation

First seed

	x_1	y_1	
	a	b	$a + b$
x_2	21354	11421	32775
	c	d	$c + d$
y_2	11421	19644	31065
	$a + c$	$b + d$	N
	32775	31065	63840

$$\chi^2 = \frac{N(ad - bc)^2}{(a + c)(b + d)(c + d)(a + b)} = \frac{63840 (289038735)^2}{(1018153375)^2} = 5144.91.$$

We require further the values of $\frac{1}{2}(1 + \alpha)$ for both variables, that is $\frac{1}{2}(1 + \alpha_1)$ and $\frac{1}{2}(1 + \alpha_2)$, in the sense in which the expression has been used by Sheppard (32).

It is merely necessary to note that in the use of the tables we must take

$$\frac{1}{2}(1 + \alpha_1) = \frac{a + c}{N} = \frac{n_{x_1 x_2} + n_{x_1 y_2}}{N},$$

$$\frac{1}{2}(1 + \alpha_2) = \frac{a + b}{N} = \frac{n_{x_2 x_1} + n_{x_2 y_1}}{N}.$$

Where $(a + c) > (b + d)$ and $(a + b) > (c + d)$. If $(b + d) > (a + c)$, $\frac{1}{2}(1 + \alpha_1) = \frac{b + d}{N}$.

In our present case the table is symmetrical and $\frac{1}{2}(1 + \alpha_1) = \frac{1}{2}(1 + \alpha_2) = .513$. Interpolating from Pearson's Table V,

$$\chi_{.05} = \chi_{.01} = 1.2536,$$

$$\sigma_{\sigma r} = \frac{1}{\sqrt{N}} \chi_{.05} \chi_{.01} = \frac{(1.2536)^2}{\sqrt{63840}} = \frac{1.571513}{252.6658} = .0062,$$

$$r_p^2 (=) \sigma_{\sigma r}^2 \chi^2 = .00003844 \times 5144.91 = .19777034.$$

$$r_p (=) .4447.$$

Turning now to the coefficients in Table III, we note that the coefficients are far larger than those given above as measures of the relationship between the total number of seedlings per subplot in the two varieties of cotton and many times as large as the intra-class and inter-class coefficients measuring the relationship between the number of seedlings per hill in the same or in adjoining subplots. Thus they indicate a high degree of localization in individual hills of conditions affecting seed germination.

The suggestion may be made that the conspicuously higher value of these coefficients is due to the method of calculation and not to the existence of factors influencing germination which are specific to individual hills.

To answer this question we have merely to determine the equivalent probability correlation between the fate of seeds in a seven-hill plot. In this case we let W represent

the total number of seeds planted in a plot, X the number germinating and Y the number failing to germinate. The frequencies for the fourfold table may again be computed from formulas (i), (ii) and (iii), the moments being derived from columns 1, 2, 4, 6 and 8 of Table II.

The actual frequencies and the values of χ^2 and r_p appear in Table IV.

TABLE IV

INTRA-CLASS EQUIVALENT PROBABILITY CORRELATION, r_p , BETWEEN FATE OF SEEDS PLANTED IN THE SAME SUBPLOT

	n_{xx}	n_{xy}	n_{yx}	n_{yy}	χ^2	r_p
Normal irrigation (Plots C3-16, C3-17)						
Pima	246974	95253	95253	86008	20144.19	.3264
Acala	151566	117189	117189	137544	5652.25	.1654
Light irrigation (Plots C3-18, C3-19)						
Pima	325908	83108	83108	31364	2623.93	.1383
Acala	164042	124516	124516	110414	774.96	.0612

The coefficients are uniformly smaller than those measuring the relationship between the fate of the seedlings of the same hill.

The coefficients are of the same order of magnitude as the intra-class coefficients for number of seedlings per hill in the same subplot.

When compared with the coefficients already discussed, these results indicate clearly that the high values of the equivalent probability correlation coefficients measuring the relationship between the fate of the seeds of the same hill as compared with the intra-class correlation for the number of seeds developing per hill in the same plot are not due to any defect in the former method. The true explanation seems to be that (a) the same physical, chemical or biological factors of the soil which influence the germination and establishment of the seedling in the

individual hills change rapidly as we pass out in the plane of space afforded by the experimental field, or that (b) there are special factors affecting the germination and establishment of the seed in the individual hills which are specific for individual hills.

In pressing this point further, we may determine the equivalent probability inter-class correlation between the fate of the seeds of Pima and Acala cotton grown on adjacent subplots. The fourfold tables may be derived from Table II by formulas (x)-(xiii). The results appear in Table V.

TABLE V
INTER-CLASS EQUIVALENT PROBABILITY CORRELATION, r_p , BETWEEN FATE OF SEEDS IN ASSOCIATED SUBPLOTS OF PIMA AND ACALA

	$n_{x_1x_2}$	$n_{x_1y_2}$	$n_{y_1x_2}$	$n_{y_1y_2}$	χ^2	r_p
Normal irrigation						
(Plots C3-16, C3-17)						
Pima with Acala	189125	161449	86185	99497	2756.47	.1155
Light irrigation						
(Plots C3-18, C3-19)						
Pima with Acala	232381	186611	63215	54049	89.39	.0227

The coefficients are of the same order of magnitude as the inter-class coefficients for number of seedlings per hill in adjoining subplots but are somewhat lower ($r_p = .1155$ as compared with $r = .1483$ and $r_p = .0227$ as compared with $r = .0411$). This is an altogether reasonable expectation when we recall that our knowledge of a relationship must become more vague as we pass from a grouping of variates recorded in numerical classes (0 to 6 seedlings per hill) into two alternative classes only.

Returning now to the problem of the correlation between the fate of the seedlings of the same subplot as set forth in Table IV, it will be apparent that these correlations represent in part the relationship between the fate of the seedlings of the same hill and in part the relation-

ship between the fate of the seedlings of different hills of the same subplot.

A further refinement of analysis may be effected by determining the correlation between the fate of seeds in different hills of a plot. The frequencies for this four-fold table are given by equations (vii), (viii), (ix). Practically the determination of these correlations requires merely the differences between the empirical frequencies of combinations as shown in Tables III and IV with the calculation of new theoretical frequencies. The results are shown in Table VI.

TABLE VI

INTRACLASS EQUIVALENT PROBABILITY CORRELATION, r_p , BETWEEN FATE OF SEEDS PLANTED IN DIFFERENT HILLS OF THE SAME SUBPLOT

	n_{xx}	n_{xy}	n_{yx}	n_{yy}	χ^2	r_p
Normal irrigation (Plots C3-16, C3-17)						
Pima	212530	87962	87962	71194	10985.55	.2515
Acala	130212	105768	105768	117900	2862.35	.1230
Light irrigation (Plots C3-18, C3-19)						
Pima	284616	74520	74520	25992	1100.14	.0962
Acala	142374	110994	110994	95286	261.49	.0372

In all four cases the values are positive. In all cases the coefficients measuring the relationship between the fate of the seedlings of the same subplot but of different hills of the subplot (Table VI) are lower than those measuring the relationship between the fate of all seedlings of the subplot (Table IV). Both of these coefficients are lower than those measuring the relationship between the fate of the seeds of the same hill (Table III). Thus it is quite clear that (a) the field is heterogeneous in the sense in which this term has been used in earlier investigations (9) (13) (24) (25), and that (b) the heterogeneity factors are so highly localized that the correlation between the fate of the seeds of the same hill is far higher than that

of the fate of seeds planted in hills more widely distributed in space. Whether certain heterogeneity factors are specific for the individual hills must be determined by further investigations.

The results for this particular experiment are not as striking as those of some which we might have chosen. They are sufficient, however, to illustrate the analytical powers of the equivalent probability intra-class and inter-class method.

IV. SUMMARY

In many cases individuals which must be classified in two alternative categories (such as male or female, died or survived) are associated for some logical reason into groups or classes. Thus, for example, a family of w children may contain x males and y females. Again, out of a series of plantings each of w seeds, x may produce plants while y may fail to germinate.

When individuals of character x and y are thus associated in classes of size w for some biological reason, the question must frequently arise as to whether such individuals of character x (or y) are distributed among the classes at random, or whether there is a statistically significant deviation from a random distribution due to some factor or factors which tend to produce a definite excess of individuals of character x (or y) in certain classes and a corresponding deficit in other classes.

Heretofore such problems have generally been dealt with by comparison of the empirical frequency distributions, or of some constant of the empirical frequency distributions, with a theoretical distribution calculated on the assumption that the distribution of individuals of a given character in the classes is due to chance only.

In this paper the serious disadvantages of such procedures are indicated. Methods of forming fourfold tables representing the possible permutations of the individuals of the same class are given in terms of the moments of the frequency distributions. This makes

possible the expression of the relationship between the character of individuals of the same class in terms of Pearson's equivalent probability correlation coefficient.

The present method of procedure has two outstanding advantages. First, it expresses the deviation of the actual distribution from the theoretical distribution, calculated on the assumption that the individuals of specified character are distributed among the classes at random, on a familiar and mentally comprehensible scale. Second, it expresses the relationship between the attributes of the individuals of the same class on a universally comparable scale, thus permitting the comparative consideration of direct, cross and fractional intra-class and inter-class correlations. This permits the more detailed analysis of many kinds of data.

While new statistical methods should always be used with caution until their validity is established by actual tests under a wide range of conditions, it may be said that the method here suggested has already been employed in the analysis of many different kinds of data without discovering any fundamental objection, and with every indication that it may contribute materially to the interpretation of the kinds of data to which it is applicable.

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THE INCIDENCE OF HUMAN MULTIPLE BIRTHS

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THE most comprehensive study of the incidence of human multiple births that was found in a rather careful examination of the literature is that of Guzzoni (1889) who in 1889 published data on more than 50,000,000 births (quoted by DeLee, 1924). He found that twins occurred once in 87, triplets once in 7,103 and quadruplets once in 757,000 cases of confinement. The statistics of G. Veit (1855), quoted by Williams (1924), which were based on 13,000,000 cases in Prussia during the period 1826 to 1849, showed that twins occurred once in 89, triplets once in 7,910 and quadruplets once in 371,125 labors. The estimate of Arey (1925), which may be considered typical of those found in modern text-books of embryology, and the findings of Guzzoni and of G. Veit are reproduced here in tabular form to aid in comparison.

Authority	Twin	Triplet	Quadruplet
Arey	1:85	1:7,000	1:750,000
Guzzoni	1:87	1:7,103	1:757,000
G. Veit	1:89	1:7,910	1:371,125

It will be noted that, with the exception of the incidence of quadruplets as given by Veit, the figures are in essential agreement.

No published record was found of any comprehensive study of recent statistics with a view to determining the relative frequency of twin, triplet and quadruplet confinements. Since such a study would be of value in making possible a comparison between the incidence of recent multiple births and the corresponding values ob-

tained by earlier investigators, it was decided to undertake it.

Acceptable data on more than 120,000,000 confinements were obtained from twenty-one countries by inquiries addressed either directly to their respective bureaus of birth registration or to the United States legation in the country concerned. It was originally intended to study these figures in all cases for the ten-year period 1916-25. Because it proved impossible to obtain such information from some of the European countries for the period of the World War, it became necessary to take whatever reliable, recent data were available, regardless of the years represented. With the exception of but one country, Belgium, where the statistics on multiple births are compiled only every fifth year, the figures used in this study are, however, for consecutive years. The number of confinements studied from each country is, moreover, sufficiently large so that the percentages of multiple births computed from them may reasonably be considered accurate representations of those phenomena as they occurred in the various countries during the period studied.

In a regrettably large number of instances, countries were either unable to supply the desired information or their method of reckoning multiple births rendered the data which they did furnish valueless for the purpose of this study. In some countries, for example, twin, triplet and quadruplet confinements are counted as such, only if the two, three or four children are born alive. If two members of a quadruplet set are stillborn, the case is recorded as a twin confinement, instead of what it very obviously is. The same method is used in reporting twin and triplet cases. The unfortunate use of the word "births" as referring in some instances to confinements and in others to the number of children born caused much delay and additional labor in checking the data. Statistics on more than 20,000,000 confinements had to be

TABLE I

Country	Years	Children born	Number of pregnancies	Twin cases	Per cent.	Triplet cases	Per cent.	Twin ratio	Triplet ratio	Quadruplet
Argentina ¹	1917-26	2,674,162	2,662,020	21,818	0.82	162	0.0060	1:122.01	1:(128.2) ²	2
Australia	1916-26	1,458,023	1,442,841	15,512	1.07	132	0.0091	1:93	1:(104.5) ²	2
Belgium	1920 and 1925	318,036	314,470	3,498	1.11	34	0.0107	1:89.8	1:(96.1) ²	0
Brazil ²	1916-25	320,659	318,443	2,172	0.68	22	0.0069	1:146	1:(120.3) ²	0
Bulgaria ³	1918-24	1,255,061	1,239,447	15,366	1.23	124	0.0100	1:80.6	1:(99.9) ²
Canada	1923-26	725,178	716,124	8,854	1.23	97	0.0135	1:80.8	1:(86.0) ²	2
Colombia ⁴	1916-22	1,150,210	1,145,406	4,657	0.40	72	0.0062	1:245.9	1:(126.2) ²	1
Denmark	1916-25	734,588	722,808	11,512	1.59	134	0.0185	1:62.7	1:(73.4) ²	2
France ⁵	1916-25	6,411,114	6,337,924	71,754	1.13	697	0.0109	1:88.3	1:(95.4) ²	14
Germany	1910-24	22,653,325	22,267,234	280,307	1.25	2,873	0.0129	1:79.4	1:(88.03) ²	46
Greece	1921-23	375,637	343,307	2,625	0.76	14	0.0040	1:130.7	1:(156.7) ²	0
Hungary ⁶	1916-25	2,635,072	2,603,344	31,124	1.19	296	0.0113	1:83.6	1:(93.8) ²	4
Italy	1872-1925	59,061,552	58,976,290	688,066	1.16	8,489	0.0143	1:85.7	1:(83.3) ²	72
Netherlands	1916-25	1,852,525	1,827,459	24,574	1.34	240	0.0131	1:74.3	1:(87.3) ²	4
New Zealand	1916-25	278,716	275,549	3,125	1.13	21	0.0076	1:88.1	1:(114.5) ²	1
Norway	1916-25	629,289	620,108	8,985	1.44	98	0.0158	1:69.01	1:(79.5) ²	3
Paraguay ⁷	1916-27	34,781	34,588	193	0.55	0	0.0000	1:179.2	0
Poland ³	1920-25	712,228	702,563	9,443	1.34	111	0.0158	1:74.4	1:(79.5) ²
Sweden	1916-23	996,238	981,584	14,384	1.46	135	0.0137	1:68.2	1:(85.3) ²	0
United States	1915-25	16,286,352	16,096,332	186,040	1.15	1,951	0.0121	1:86.5	1:(90.8) ²	26
Uruguay	1916-26	438,532	433,557	4,903	1.13	36	0.0083	1:88.4	1:(109.7) ²	0
Total		121,001,878	120,061,398	1,408,912	1.17	15,738	0.0131	1:85.2	1:(87.3) ²	179

¹ Statistics do not include Entre Rios for the years 1917, 1918 and 1924, or Córdoba, Jujuy and Salta for 1918.

² Data on multiple births were available for the City of Rio de Janeiro only.

³ No data on quadruplets obtainable.

⁴ Statistics for 1920, 1921 and 1922 do not include figures for the Departamento de Bolívar.

⁵ Data for the period 1916-19 cover seventy-seven departments. Those for the years 1920 to 1925 are for ninety departments.

⁶ Figures for 1916, 1917 and 1918 are those of the pre-war territory of Hungary. Those for the remaining years cover only the present territory of Hungary.

⁷ Statistics were available for the city of Asunción only.

omitted from this study because of these or other similar inaccuracies.

All acceptable data were carefully studied and the desired values computed from them. The figures so obtained are summarized in Table I, in which the countries studied are arranged in alphabetical order. For each country are given, in successive columns: the years studied; the total number of children born; the total number of confinements; the total number and average percentage of twin confinements; the total number and average percentage of triplet confinements; the twin ratio; the triplet ratio, and the total number of quadruplet confinements, if any. The last line of the table contains corresponding values for all the countries studied taken together. Appendix A gives in the case of each of the countries the exact source from which the statistics were obtained.

Fig. 1 is a graphic representation of the twin percentages for each of the countries from which acceptable data were received. In it the figures are arranged in the order of their respective magnitudes, the one representing the country with the highest twin percentage being first. It will be noted that the twin percentage varied from 1.59 per cent. in the case of Denmark to .40 per cent. in Colombia. It seems desirable to mention that the average twin percentage listed for each country is not the mean of values which differ widely from year to year; on the contrary, the yearly twin percentages fluctuated within surprisingly narrow limits in the case of each country studied.

In practically every instance, however, the triplet percentage varied greatly from year to year. Unlike the average *twin* percentage, therefore, the average *triplet* percentage for each country is, in most instances, the mean of a number of widely different values. The average percentage of triplet confinements for the years studied was highest in Denmark (.0185 per cent.) and lowest in Greece (.0040 per cent.).



FIG. 1. The average twin percentages of the various countries for the years studied.

Table II lists the average twin and the average triplet percentages for each of the countries studied, the name of the country having the highest percentage being listed first in each instance. It will be noted that, while the various countries do not all have exactly the same position in both columns, nine of the ten countries highest in the list of average twin percentages are found also among the first ten places in the list of average triplet percentages. This fact suggests the existence of some relationship between the incidence of these two types of multiple births, another indication of which may now be discussed.

TABLE II

	Average twin per- centage	Country*	Country*	Average triplet percentage	
1	1.59	Denmark	Denmark	0.0185	1
2	1.46	Sweden	Norway	0.0158	2
3	1.44	Norway	Poland	0.0158	3
4	1.34	Netherlands	Italy	0.0143	4
5	1.34	Poland	Sweden	0.0137	5
6	1.25	Germany	Canada	0.0135	6
7	1.23	Bulgaria	Netherlands	0.0131	7
8	1.23	Canada	Germany	0.0129	8
9	1.19	Hungary	United States	0.0121	9
10	1.16	Italy	Hungary	0.0113	10
11	1.15	United States	France	0.0109	11
12	1.13	France	Belgium	0.0107	12
13	1.13	New Zealand	Bulgaria	0.0100	13
14	1.13	Uruguay	Australia	0.0091	14
15	1.11	Belgium	Uruguay	0.0083	15
16	1.07	Australia	New Zealand	0.0076	16
17	0.82	Argentina	Brazil	0.0069	17
18	0.76	Greece	Colombia	0.0062	18
19	0.68	Brazil	Argentina	0.0060	19
20	0.40	Colombia	Greece	0.0040	20

* The names of the countries studied arranged in the order of the magnitude of their respective average twin and triplet percentages.

Zeleny (1921) reported what he considered to be a previously undescribed relationship between the number of human twin and triplet confinements. To quote him:

If $1/n$ is the proportion of twin births to all births in a large population during any period, then the proportion of triplet births during the same period is very near to $1/n^2$.

Attention had previously been called to the same relationship by Edgar (1916), who in commenting upon Strassman's estimate of the twin and triplet ratios in Germany, wrote as follows:

The ratio of triplet, twin and ordinary labors in Germany is given by Strassman as 1:89:7,921. It is of interest to note that in this series the number of twin pregnancies is exactly the square root of the number of single births.

Zeleny (1921) points out that the observed relationship between the incidence of various types of human multiple births is explainable on the assumption that triplets are produced by the coincidence of two, quadruplets by the coincidence of three, independent processes occurring with equal frequencies. According to his view, one of these processes by itself gives rise to twins.

On consulting the totals in Table I it will be seen that there was one set of twins born out of each 85.2 confinements, and that there was one set of triplets for each 7,628.75 cases. The total twin and triplet ratios are respectively 1:85.2 and 1:7,628.75 or 1:(87.3)². There was, moreover, one set of quadruplets for every 670,734 confinements, the ratio being 1:(87.5)³, a very close approach to the values expected on Zeleny's hypothesis that, if twins occur once in n confinements, triplets and quadruplets would occur respectively once in n^2 and once in n^3 confinements.

Considering the large number of cases included in this study and the many factors which militate against obtaining absolutely accurate statistics, it is thought that these data must be considered as confirming the existence of the relationship between the number of human twin, triplet and quadruplet confinements to which attention has been directed by Edgar and Zeleny.

Results more closely approaching the theoretical are scarcely to be expected; indeed, as will appear from the

following discussion, the almost ideal figures cited by Edgar (1916) and by Zeleny (1921) are in all probability based on faulty, though authentic, statistics.

If Zeleny's hypothesis has any basis in fact, *it must of necessity apply to conceptions and not to confinements*. The latter frequently differ from the former both in number and in kind, in the case of multiple pregnancies. DeLee (1924) estimates that about 70 per cent. of twin pregnancies end before term and that practically all triplet and quadruplet gestations do so. A certain percentage of multiple pregnancies, especially triplet and quadruplet ones, are interrupted so early in their course that the children are stillborn. In many parts of the world—in some states of this country for example¹—non-viable products of a gestation of less than six, seven or even eight months' duration are not reportable either as births or as deaths, and so completely escape registration. There are, in addition, those multiple pregnancies which are marked by the death of one or more embryos early in gestation, due to hazards which undoubtedly increase with the number of embryos in the uterus. Such confinements are in every instance erroneously classified, because the number of individuals eventually born from them is smaller than the number originally conceived. These and other similar sources of error could well explain those instances in which the number of triplet and quadruplet confinements is smaller than that expected according to Zeleny's hypothesis. They explain, too, why statistics exactly fitting the hypothesis (if it be the correct one) are neither to be expected nor desired.

In seventeen of twenty countries from which data on both twin and triplet confinements were received, the recorded number of triplet cases was in each instance less than that to be expected according to the view under discussion, and the differences may be reasonably attributed

¹1925. "Births, Stillbirths and Infant Mortality Statistics for the Birth Registration Area of the United States, 1923," Government Printing Office, Washington, p. 21.

to sources of error such as are described in the last paragraph.

In three other countries, however, Brazil,² Colombia and Italy, the number of triplet confinements reported is *larger* than that expected from the recorded cases of twins. The difference in each of the first two countries will be seen to be very marked, and, if the statistics on which they are based are accurate, it is difficult to see how they can be reconciled with Zeleny's hypothesis. An attempt is being made to obtain birth statistics from these two countries for other years, in order to determine whether or not the observed relationship between the number of twin and triplet confinements is constantly different from that expected.

The case of Italy is not quite the same. The statistics of that country which are included in this study cover the period from 1872 to 1925. The discrepancy appears in the figures for the interval between 1872 and 1911. For those years the recorded number of triplet confinements is larger than would be expected on the basis of the number of twin pregnancies reported. In more recent statistics from the same country, however—in those for the period 1912-25—the number of triplet confinements is smaller than the theoretical and, therefore, is explainable on the basis of the hypothesis under discussion. In view of this fact, it seems quite possible that the earlier statistics may be faulty.

Assuming that the completeness of birth registration in Italy has increased with the years, the discrepancy noted in the older data might be accounted for on the grounds that formerly triplet births, because of their relative rarity, were so widely discussed that they were much more likely to come to the attention of registering officials than were the less remarkable twin confinements. Though such an assumption by no means proves the case, its reasonableness and the orthodox behavior of the twin

² Available data from Brazil covered only the Federal District, the city of Rio de Janeiro.

and triplet ratios in the later (and presumably more reliable) Italian statistics strongly suggest the inaccuracy of the earlier data. In any case, as stated above, the weight of evidence disclosed by this study decidedly favors Edgar's and Zeleny's view as to the relative frequency of the various types of human multiple gestations.

SUMMARY

1. Statistics on more than 120,000,000 cases of labor were obtained from official sources in twenty-one countries, and the percentages of the various types of multiple births were computed from them.

2. The percentage of multiple births was found to vary greatly for different countries. The twin percentage ranged from 1.59 per cent. in Denmark to .40 per cent. in Colombia, and the triplet percentage from .0185 per cent. in Denmark to .0040 per cent. in Greece.

3. Twin confinements were found to occur once in 85.2, triplet confinements once in 7,628.7 and quadruplet confinements once in 670,734 cases of labor in this study covering 120,061,398 pregnancies. These results agree essentially with findings of the earlier and less extensive investigations of Guzzoni and of G. Veit and also with the estimate of Arey. This agreement seems to indicate a fixed relationship between the incidence of the various types of human gestations.

4. A general agreement was noted between the twin and triplet percentages in the individual countries. Those countries having a large twin percentage had also a large triplet percentage, while those reporting few cases of twins reported also few cases of triplets.

5. The total twin, triplet and quadruplet ratios were found to be respectively 1: 85.2; 1: (87.3)², and 1: (87.5)³. These values very closely approach those expected according to Zeleny's hypothesis that, if $1/n$ is the proportion of twin births to all births in a large population, then the proportion of triplet births and the proportion of

quadruplet births for the same period will approximate respectively $1/n^2$ and $1/n^3$.

In conclusion, the writer wishes to acknowledge his indebtedness to the officials of the various countries whose cooperation in furnishing authentic statistics made this paper possible. Especial thanks are due, also, to Professor Ira E. Cutler, of the University of Denver, under whose direction this study was begun, and to Professor Francis Ramaley, of the University of Colorado, whose encouragement and helpful suggestions have aided greatly in the preparation of the manuscript.

APPENDIX A

<i>Country</i>	<i>Source from which statistics were obtained</i>
Argentina	Secretary of the National Department of Hygiene of Argentina, 1928.
Australia	Commonwealth Bureau of Census and Statistics, Melbourne, January 16, 1928.
Belgium	Year Book of Statistics for Belgium, Vol. L, p. 44.
Brazil	Brazilian National Board of Health (through the American Consul General, Rio de Janeiro, March 16, 1928).
Bulgaria	Secretary of the U. S. Legation, Sofia, January 5, 1927.
Canada	Dominion Bureau of Statistics, Ottawa, December 20, 1927.
Colombia	American Minister, Bogota, January 23, 1928.
Denmark	Statistical Department of the Danish Government (through the U. S. Legation at Copenhagen, December 31, 1927).
France	Statistique Générale de la France (through the French Ambassador to the United States, Washington, February 8, 1928).
Germany	Statistisches Jahrbuch für das deutsche Reich, 1916, Vol. 37, p. 7, and 1924, Vol. 44, pp. 36-41.
Greece	Foreign Office of Greece (through the U. S. Legation, Athens, January 30, 1928).
Hungary	Royal Hungarian Bureau of Statistics (through the U. S. Legation, Budapest, January 11, 1928).
Italy	Movimento Della Popolazione Seconde Gli Atti Dello Stato Civile Nell'Anno 1925, Rome, 1927, p. 67.
Netherlands	Jaarcijfers voor Nederland, 1925-26, p. 17.
New Zealand	Census and Statistics Office, Wellington, January 6, 1928.
Norway	Bureau Central de Statistique, Oslo, February 14, 1928.
Paraguay	National Statistical Office of Paraguay (through the American Vice Council, Asunción, March 12, 1928).
Poland	Kwartalnik Statystyczny, Rok 1927, Tom IV Zeszyt 2, Warsaw, 1927, p. 382.
Sweden	Bureau Central de Statistique de Suede, Stockholm, December 31, 1927.

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THE STIMULUS TO THE BREEDING MIGRATION OF THE SPOTTED SALAMANDER,
AMBYSTOMA MACULATUM (SHAW)¹

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THE early spring migration activities of the spotted salamander, *Ambystoma maculatum* (Shaw), were studied by the writer at White's Woods,² Ann Arbor, Michigan, in 1924 and 1926, and the tentative conclusion reached that the salamanders come to the breeding pond on the first night when the temperature is above freezing, following the first warm day in early spring. Observations were made principally after dark, with the aid of an acetylene headlight, because only then were the salamanders found active. The animals were more or less sensitive to strong light, but could be watched by weak light without being disturbed.

With the above-mentioned theory in mind, the same pond was visited in 1927 on the first night above freezing, March 10, following a maximum temperature during the day of about 54° F. Contrary to expectation, there were no salamanders in the pond. The pond was full of ice except for a very little water in places at the edge, but the ground was free from snow. On the following night, also, there were no salamanders, although the temperature was 45°. On this evening there was a little more free water around the edge of the pond, and over the floor of the woods the superficial leaves, earth and logs were free from ice. On the evening of March 12, temperature 54°, three males were found in the pond and

¹ Contribution from the Zoological Laboratory of the University of Michigan.

² White's Woods is a small tract of upland deciduous woods, mostly oak, lying just west of the city of Ann Arbor, and owned by the University of Michigan. The pond where the salamanders were observed is about two hundred feet long and forty feet wide, and probably not more than three feet deep.

one under a log about thirty-five feet away. On the next morning, March 13, there was one group of spermatophores. There was now free water all around the edge and the ice was fast disintegrating. On succeeding evenings through the 17th there were no more spermatophores, but on the evening of the 13th three males were found and two females; on the next evening five males, one female and two of undetermined sex; on the evening of the 15th four males and one female; on the evening of the 16th five males and one of undetermined sex; on the evening of the 17th four males. On the evening of the 18th thirty males and seven females were taken and three others were seen. In addition there were ten groups of fresh spermatophores.

Thus the first salamanders were found on the evening of the 12th, and on this night a limited amount of breeding activity occurred. On succeeding nights through the 17th there was no appreciable addition to the salamander population of the pond and no more breeding. (Male salamanders were returned to the pond and females were saved for further study.) The temperatures recorded at Ann Arbor for the Weather Bureau³ for this period are shown in the table on page 156.

From this it seems clear that the maximum and minimum temperatures had nothing to do with the arrival and breeding of these salamanders on March 12 and 17.

If, however, we consider the incidence of rainfall in connection with the observations on the activities of the salamanders an interesting correlation will be observed. There was no rain on the 10th or the 11th and there had been none on the two preceding days. Early in the morning of the 12th there was a little rain and in the afternoon rain fell until dark, and it was on this evening that the first salamanders were found. In the latter part of the night of the 12th considerable rain fell and it was on the

³ Temperatures for 1926 and 1927 are taken from the Climatological Data of the U. S. Dept. of Agriculture, Michigan Section, vols. 41 and 42, nos. 3; publ. at Lansing, Michigan.

Date (1927)	Max. Temp. (Fahrenheit)	Min. Temp. (Fahrenheit)	
March 9	43	25	
" 10	51	25	
" 11	63	35	
" 12	59	43	First appearance; first breeding
" 13	58	50	} No increase in salamander population in pond
" 14	50	39	
" 15	56	41	
" 16	70	31	} Second breeding late this night
" 17	62	45	
" 18	60	42	
" 19	54	35	
" 20	37	28	
" 21	38	31	
" 22	40	28	
" 23	39	22	
" 24	45	27	
" 25	50	37	Third breeding late this night
" 26	43	35	
" 27	38	31	
" 28	42	30	
" 29	48	26	

next morning that there was the first evidence of breeding—a group of seventeen fresh spermatophores. From this time until the 17th there was no more rain. In the morning of the 17th a light rain fell, and in the night, after the observations for that evening had been taken, there was a drenching rain. On the following evening, the 18th, salamanders were first found to be numerous in the pond, many fresh spermatophores were present and breeding behavior was observed.

On the six following days, March 19 to 24, the temperature was below freezing at night and the pond was not visited. (The record of the Weather Bureau, however, shows a minimum of 35° for the 19th.) On the evening of March 25 the temperature was above 37°. No spermatophores were present in the pond (all those deposited earlier had disappeared), and only ten salamanders were seen. A light rain started about 8:30 p. m. and developed into a hard rain later in the night. On March 26 the temperature was 35° in the evening. At this time sixty-two males were seen, thirteen females and seventeen of undetermined sex; and fifty-seven groups of fresh

spermatophores were counted. Mating activity was in progress at several of these groups.

The facts thus far recounted for the period March 10 to 26, and summarized in the accompanying table, are subject to the following interpretation: These salamanders migrate to the breeding pond (1) almost exclusively at night, (2) only when the temperature is above freezing and (3) only during a rain. The most effective rain in bringing the salamanders to the pond is a hard rain early in the night or a rain prolonged during the whole night. Presumably all the salamanders begin the migration at about the same time, but those unable to reach the pond during the continuance of favoring conditions go into hiding wherever overtaken by the unfavorable conditions, such as daylight or freezing temperatures. Thus, if a rain is sufficient to bring only a part of the salamanders to the pond, the remainder will wait for the next favoring rain. The application of this theory to the writer's data for 1924 and 1926 will now be considered.

In 1926, on the first mild evening of the season, March 24, the temperature was 45° F. The pond in White's Woods was almost full of ice, but in places at the south end there was free water for a space of a foot or more. In this bit of free water the writer collected many isolated males and seventeen more crawling on the ice. Some of the latter were close to the shore; others were from several feet to as much as twelve feet out. One was crawling on the snow close to shore. Some of those on the ice were quiet, some were crawling slowly, some were resting with their tail ends in shallow, wet depressions in the ice. At the north end of the pond where there was a space of about three feet of open water, six salamanders were together about two feet from shore. They were moving in and out and over and under each other, more actively than usual for the species, but not in any excitement and without evidence that they were seeking each

OBSERVATIONS ON SALAMANDERS IN POND IN WHITE'S WOODS, 1927

Date	Time of visit	Temperature (Fahrenheit)	Precipitation	Males	Females	Males or females	Groups of fresh sper- matophores
March 10	Evening	Above freezing	None	0	0		0
" 11	"	45	"	0	0		0
" 12	"	54	In early morn- ing and in afternoon				
" 13	Morning	54-58	Much rain lat- ter part of last night	3	0		0
" 13	Evening	44	None	3	2		1
" 14	"	42	"	5	1	2	0
" 15	"	34	"	4	1		0
" 16	"	45	"	5	0	1	0
" 17	"	41	Light rain in morning only				
" 18	"	43	Sharp rain last night after return from pond	4	0		0
" 19-24		Below freezing					
" 25	Evening	Above 37	Light rain start- ing about 8:30 P. M.	30	7	3	10
" 26	"	35	Hard rain pre- ceding night	6	1	3	0
				62	13	17	57

other. They were in the midst of numerous very white, fresh-appearing spermatophores that covered a space of about a foot in diameter. One of these salamanders was evidently a female, as she was much larger and plumper. She was distinctly seen to stop over a spermatophore and rest with her cloaca over it for several seconds, then to move away. At times others stopped and rested several seconds with the hind legs somewhat stiffly back and to the sides; and when the animal moved away a spermatophore appeared just where the cloaca had been. A male that was picked up just after one of these performances had the cloacal lips spread and in motion. All the salamanders in this group were collected, and later examination showed them to be five males and one female.

These observations of March 24, 1926, were made between 9:00 and 10:00 P. M. The moon was shining. A sharp thunder shower occurred between 7:00 and 8:00 P. M. (0.07 inch),⁴ and the floor of the woods was thawed to just under the superficial leaves. The next morning everything was tight with frost. The next evening, March 25, the temperature was 32° and a thin coat of ice covered what had been the free water at the margin of the heavy ice. Ten males and six females were collected. There were no breeding groups, but many clusters of spermatophores in the pond showed breeding since 10:00 P. M. the night before.

This experience of 1926 is in keeping with the theory deduced from the 1927 observations. But in 1926 the significance of the sharp thundershower early in the evening was overlooked, because at that time the rain came on the first warm night and the writer was thinking only of a temperature correlation.

The observations in 1924 were incomplete but significant in connection with the present theory. The temperatures recorded for the Weather Bureau covering the period of interest are as follows:

⁴ Record of Detroit Observatory of the University of Michigan kindly furnished by Professor R. H. Curtiss.

Date (1924)	Max. Temp. (Fahr.)	Min. Temp. (Fahr.)
March 26	39	34
" 27	54	30
" 28	51	35
" 29	56	32

On the night preceding March 26 there was snow and rain. Rain began at 3:30 p. m. and ended during the night, with a total precipitation of 0.05 inch. On March 27, the first mild day, the pond was largely covered with ice. The temperature was above freezing that evening, but the pond was not visited. There was no rain on March 28 until 8:30 p. m. At this time the pond was found to be free from surface ice, except slush in a few places, but there was still much ice over the bottom. Many logs in the woods were frozen tightly to the ground. Nine salamanders were collected in the pond. There was no breeding, but a cluster of about six spermatophores showed there had been one breeding group. The temperature was 38° and there was a light rain. These salamanders could hardly have reached the pond and deposited spermatophores on this evening, so it is to be presumed that they came on the night before March 27, when occurred the first rain which they could use. The rain which began lightly at 8:30 this evening (March 28) increased in amount and lasted until the next morning when a total of 0.41 inch was recorded by the Observatory. Then from 9:30 a. m. to 4:30 p. m. on March 29, 0.18 inch more fell. On this evening between 8:00 and 9:00 p. m. with the temperature at 40°, the writer collected sixty-five salamanders (fifty-nine of them males) and found numerous groups of spermatophores in the pond. Thus, clearly, for this season the heavy migration and breeding occurred in coincidence with the first heavy night rain.

The observations by the writer are thus in agreement with the theory that these salamanders require a rain at

night for the breeding migration.⁵ Once started, however, the salamanders presumably continue in movement during the night until the ground becomes uncomfortably dry.

Twenty years ago this subject was investigated by Albert H. Wright and A. A. Allen,⁶ at Ithaca, New York, and conclusions different from those above stated were reached.

From casual observations over a period of several years Wright stated that "it is obvious that a temperature of 50° F. or more almost invariably caused the species to emerge" from hibernation, and he considered that his careful records of 1908 supported this conclusion. Let us consider these observations separately in the light of the new theory and in connection with the temperature and precipitation records of the Ithaca station of the United States Weather Bureau.⁷ Wright gives first appearances from hibernation for this species as follows:

1903, March 13; 1904, April 1; 1905, April 1; 1906, March 28; 1907, March 24; 1908, March 23. It may perhaps fairly be asked at this point, if, by casual observations or even by very careful observations, absolutely reliable records of the first appearances of a species can be obtained. It should be remarked, also, that some of these first appearances may be based on individuals discovered before they had begun the migration movement.

⁵ Since the above was written an article on "The Courtship of the Spotted Salamander" by Ruth B. Breder (*Bull. New York Zool. Soc.*, vol. 30, no. 3, May-June, 1927) has come to hand. It is noteworthy that the extensive breeding herein described occurred on a rainy night, and on this night alone out of ten or eleven successive nights on which the pond was regularly visited.

⁶ "The Early Breeding Habits of *Amblystoma punctatum*," *AMER. NAT.*, vol. 43, pp. 687-692, 1909. Preliminary results on this subject were published by Wright in 1908 in a paper entitled "Notes on the Breeding Habits of *Amblystoma punctatum*," (*Biol. Bull.*, vol. 14, no. 5, pp. 284-289).

⁷ The records given here are taken from the carbon copies on file at the Weather Bureau in Washington, D. C., to which the writer was given free access through the kindness of William F. Clark, of the Weather Bureau.

However, to consider the first case—the Weather Bureau records^s from March 7 through March 13, 1903, are as follows:

Date (1903)	Precipitation	Max. Temp.	Min. Temp.
March 7	T	46	32
" 8	.44	48	37
" 9	T	43	34
" 10	.05	49	41
" 11	.38	47	38
" 12	0	49	35
" 13	T	52	31 First appearance

The rain on the 11th was in the daytime; that on the 13th was during the night, presumably the night before. The appearance of the salamanders on the 13th was due, according to the writer's theory, to the rain of the night before.

For 1904 the Bureau records are as follows:

Date (1904)	Precipitation	Max. Temp.	Min. Temp.
March 22	0	47	33
" 23	.38	48	33
" 24	0	57	27
" 25	.09	58	47
" 26	.08	59	26
" 27	T	30	23
" 28	T	30	19
" 29	0	41	18
" 30	0	50	32
" 31	.08	40	36
April 1	.26	53	36 First appearance

The rain of April 1 began during the preceding night and continued during that day and a part of the following night. The rain of March 31 was in the daytime. These records show that, while salamanders might have been expected on March 24, 25 or 26, unless there was snow on

^s Precipitation is given in inches (T means trace); temperatures are given in Fahrenheit.

the ground, there was otherwise no logical date, according to the writer's theory, except April 1, the date they were first found.

In 1905 the first dates that need be considered show the following records:

Date (1905)	Precipitation	Max. Temp.	Min. Temp.
March 23	0	47	37
" 24	.40	48	38
" 25	.05	53	34
" 26	.01	64	32
" 27	0	58	44
" 28	0	76	36
" 29	0	82	52
" 30	.18	65	43
" 31	0	64	37
April 1	0	43	31 First appearance

If temperature brings the species out it should have been found on any of the above dates in March, except perhaps the first two. On the writer's theory the salamanders should have been found on March 25, following the rain that lasted all the preceding night, unless, as may well have been the case, the ground was too tightly frozen or was covered with snow. The rain of the 30th was a daytime rain that lasted until after dark. On the writer's theory, the species could have been found a day or two earlier than the first of April. On Wright's theory it is difficult to account for the first appearance on April 1.

In 1906 there was still four inches of snow on the ground on March 25 and a trace of snow was recorded through the 30th. The Weather Bureau records follow:

Date (1906)	Precipitation	Max. Temp.	Min. Temp.
March 25	0	37	8
" 26	0	43	25
" 27	T	51	32
" 28	T	34	31 First appearance

Wright gives for the 28th the temperatures recorded by the Weather Bureau for the 27th—or perhaps he intended to give the 27th as the date of first appearance. This case may be interpreted by either theory.

For 1907 the figures, succeeding precipitation as snow, are as follows:

Date (1907)	Precipitation	Max. Temp.	Min. Temp.
March 21	0	50	20
" 22	0	50	43
" 23	0	61	43
" 24	T	54	33 First appearance
" 25	0	56	32
" 26	T	64	38
" 27	.12	70	45 Crest of migration

The trace of rain on March 24 fell during eight minutes in the daytime and thirty minutes in the early part of the night. The trace on the 26th was in the morning. The rain of the 27th fell during the latter part of the preceding night and the late afternoon and evening of the 27th. These facts fit the writer's theory very nicely.

For 1908 Wright's figures and those of the Weather Bureau are as follows:

Date (1908)	Precipitation	Max. Temp.	Min. Temp.	Number of salamanders collected in migration
March 23	.01	47	42	First appearance
" 24	T	49	22	1
" 25	0	37	19	
" 26	0	65	37	15
" 27	.01	63	36	22
" 28	.07	72	41	55
" 29	.31	41	30	
" 30	0	48	33	
" 31	.04	43	39	
April 1	.03	48	27	17
" 2	.02	43	25	
" 3	.04	30	23	
" 4	T	32	19	3
" 5	.15	50	18	
" 6	.01	62	40	1

The rain on March 23 was from 1:15 to 5:15 in the afternoon and on this date salamanders were first seen; that on the 24th was in the evening and at this time one individual was found. The rain on the 27th was in the early evening, that on the 28th was at various "intervals"; but that recorded for the 29th occurred during the preceding night. The whole period is notable for many small rains. This migration well supports the theory of stimulation by temperature, but may, with the exception of the salamanders collected on March 26, quite as well be interpreted on the theory of rain causation.

In addition to the above observations Wright gives the dates April 21 and 22 for the migration "crest" in 1901. The Bureau records show that this can be very well explained on the rain theory. During the period from the 19th through the 25th of April rain fell almost continuously. There was a trace on the 18th. From the 11th to the 17th there was no rain, but the maximum temperatures were all about 60° and the minimum near 38°. There was a slight rain on the 10th. Preceding this were two days of snow, three of cold rains, two of snow, four of no precipitation and two more of snow. Possibly salamanders could have been found on March 24 to 26, but there are no records.

Some of these observations given by Wright and Allen may be interpreted on the theory of temperature causation that they propose; others can not be so interpreted; and practically all are explainable, in the view of the writer, on the basis of the theory proposed in this paper.

DISCUSSION AND SUMMARY

The facts presented seem to show that migration to the breeding ponds depends, in this species, on rain and not on temperature. A winter rain coming before any thawing of the surface of the ground or while the ground is covered with snow will, of course, have no effect on the salamanders. But as soon as the surface of the ground in the woods is largely freed from snow and ice and

slightly thawed, a rain at night will bring the salamanders out, whether or not the ponds are free from ice. In general, however, if the weather has been mild enough to melt the snow and thaw the surface of the ground there will be enough free water at the edges of the ponds for the salamanders to get into the water. A rain in the daytime will have little, if any, effect; but such a rain prolonged until evening will allow some of the animals to reach the ponds. A hard rain in early evening or prolonged during the night will allow many salamanders to reach the ponds, because there will be time for the more distant individuals to crawl the intervening distance. A warm rain should be more effective than a cold one, because such a rain would thaw the ground more deeply and thus set free more salamanders. If migration occurs in the daytime it is probably only when the clouds are very dense and the dusk of evening is coming on.

According to observations by Wright and Allen at Ithaca in 1908⁹ the males begin the migration and precede the females to the pond by a day or two. The writer's observations on March 24, 1926, described above, confirm this conclusion to a certain extent. On this date the pond was visited very soon after the first salamanders had reached it, and among seventeen individuals found wandering on the ice and thirteen others in the water at the edge of the ice, there were no females. However, one female had reached the pond at this time and was already surrounded by five males that were actively depositing spermatophores; and on the following evening six females were collected in the water and many groups of spermatophores were present in the pond. This leads the writer to infer that the same stimulus starts the migration of all adults of both sexes, but that the males respond to the rain more quickly and move more rapidly, and so, on the average, reach the pond in advance of the females. That the males in fact are more active at this season than the females is shown by their behavior in the water. Here the salamanders may be seen at night mov-

⁹ AMER. NAT., 43: 689.

ing about or resting above the leaves, and collections of such individuals always results in a much larger number of males than of females. But that females are really plentiful is shown by the large numbers of egg masses to be found a few days later. This disproportion of male salamanders in such collections is probably due to the same reason as that male frogs and toads are easier to find at the breeding season, *i.e.*, because the males are the seekers and singers.¹⁰ Some females will chance to be near the pond and so will arrive with the first males. This has been the experience of the writer.

After the females reach the water there is probably not much delay in mating, for the writer has yet to collect from the water an unmated female. Of all the females that were found in White's Woods pond in 1927, twenty-four in number, one had laid all her eggs, another had laid most of them, and the other twenty-two individuals laid eggs in the laboratory. Only one of these laid infertile eggs and she appeared abnormal in other respects.

In summary we may say then that in such a climate as that of southern Michigan the spotted salamanders begin migration to the breeding ponds during the first rain at night following disappearance of the snow and thawing of the surface of the ground in the woods; and that a prolonged rain, or several rains, will be required to bring all the adult individuals of the species to the breeding sites.¹¹

¹⁰ In keeping with the idea that the two sexes are equally numerous is the result of a count of all the transformed sexually immature individuals collected by the writer at various times over a period of several years. There proved to be seventeen of each sex.

¹¹ The author has kindly given me an opportunity to read his manuscript. Time is not at hand to re-examine the details of our study of twenty years ago, but my experience in many parts of the country since then has shown clearly the great importance of rain, moisture and humidity as factors in the breeding of amphibians. The significance of rain at Ithaca is perhaps not very evident, as a rule, because here the spring is quite continuously humid from rain, thawing snows and water running off the hills and down the ravines. Temperature, as a factor, is usually secondary, but on occasions may be of much importance. I regard the most necessary condition for each species as humidity above a more or less specific minimum temperature.—A. H. WRIGHT.

THE OPAH OR MOONFISH, *LAMPRIS LUNA*,
ON THE EASTERN COAST OF
NORTH AMERICA

DR. E. W. GUDGER
AMERICAN MUSEUM OF NATURAL HISTORY

ABOUT two years ago I recorded the coming ashore on the west coast of Florida of a fine four-foot specimen of the opah. Recent information has come to me of the capture on the New England coast of two other specimens of this rare and beautiful fish. So little is known of it and so few are our records of its occurrence that it seems well worth while to make note of these two—especially as an excellent figure is at hand of one of these fish—and briefly to refer to the other captures.

The earlier of the two records was first made by Mr. Frederick F. Dimick, secretary of the Boston Fish Bureau. In his *Report* for 1925,¹ he writes under the heading, "An Unusual Fish Landed at Boston":

The schooner *Falmouth* (Captain Jeffrey Thomas) engaged in fishing for halibut, arrived July 27, 1925, and landed at the Boston Fish Pier a specimen which was identified as an Opah, Moonfish, or Jerusalem Haddock. It was about three feet long and weighed about 110 pounds. The fish was captured on the southeastern part of Western Bank in deep water, west-south-west from Sable Island.

Photographs of this fish were sent to the U. S. Bureau of Fisheries in Washington, and from them the fish was identified by Mr. Lewis Radcliffe, deputy commissioner of fisheries. In *Copeia* for February 25, 1926,² he has a note putting this capture on record in a scientific journal.

The other fish was taken by Mr. Robert A. Goffin, biological collector of the laboratory of the U. S. Bureau of Fisheries at Woods Hole, Massachusetts, on September 17, 1928. Mr. Goffin writes that "the fish was taken

¹ Boston, January, 1926, pp. 2-3.

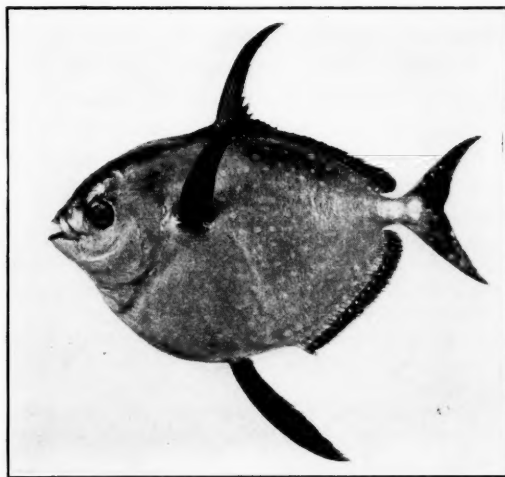
² No. 151, p. 112.

alive at Grand Island near Point Gammon, Hyannis, Mass., where it was stranded at low tide on the beach in a gully where some excavating was being done."

Dr. Francis Harper, secretary of the Boston Society of Natural History, after noting the capture of this specimen goes on to say:³

Through the interest of Mr. Malcom G. Chase, of Hyannis, and Mr. George M. Gray, of the Marine Biological Laboratory at Woods Hole, the fish was presented to the museum [of the society]. Its flat, deep form, its scarlet fins and a brilliant combination of body colors give it a striking and beautiful appearance. The species lives in the open waters of the Atlantic and the Pacific, and this seems to be the first definite record for it in New England shore waters.

In the next issue of the *Bulletin*, Dr. Harper gives as a frontispiece an excellent reproduction of the mounted fish as it hangs on the wall of the fish room of the society's museum. Through Dr. Harper's courtesy I am able to reproduce this figure herein from a photograph sent me.



—By courtesy of Dr. Francis Harper

FIG. 1. Opah taken at Hyannis, Massachusetts, September 17, 1928. Photograph of the mounted specimen in the museum of the Boston Society of Natural History.

³ *Bulletin Boston Society Natural History*, 1928, 49, pp. 17-18.

The fish reached Boston in excellent condition, and Dr. Harper took pains to make careful measurements and record the colors while the fish was fresh. For a transcript of these, and for the other information given me, I am under many obligations to Dr. Harper.

The total (outside) length was 805 mm (31.75 in.); the standard length (to the base of the caudal fin) was 688 mm (27.1 in.). The depth (body only) was 425 mm (16.75 in.); of caudal peduncle, 50 mm (2 in.). The head was 216 mm (8.5 in.) long, and the large eye 50 mm (2 in.) in diameter. The dorsal fin had forty-nine rays, and the anterior part was 258 mm (10.1 in.) high. The pectoral fin with its twenty-one rays was 210 mm (8.25 in.) in length. This was exceeded by the pelvic with its fourteen rays which measured 300 mm (11.8 in.). The lateral-line scales were very small and hard to count, but they numbered approximately eighty-six. The mouth was small and toothless, the maxillary being only 70 mm (2.8 in.) long. The premaxillary is protractile. The gill rakers number $14 + 2$. The fish weighed thirty-two pounds.

The opah is remarkable for its brilliant colors. These have, I believe, never been noted for any fresh American specimen, and it seems well to record Dr. Harper's carefully made notes, exactly as he communicated them.

Fins between scarlet and Brazil red: ventral tipped with Martius yellow; dorsal and anal narrowly bordered with same; caudal more broadly bordered with same on posterior margin. Pectoral with pale narrow posterior border. A few pale bluish spots along base of dorsal and anal.

Body color varies from deep olive to dark olive, darkest above. Numerous pale spots varying from 5 to 15 mm in diameter. A purplish (deep hellebore red) luster on dorsum and posterior portion of venter. Skin smooth except on posterior half (especially from the lateral line downward), where roughly scaly. Iris a sort of pale bronze. Cheeks and area below pectoral with decidedly silvery luster. Jaws reddish or vermilion (about same color as fins).

So far as I can find only three previous captures have been noted for our eastern coast, plus one from Gulf

waters. The first record is from the pen of that veteran ichthyologist, Dr. David Starr Jordan.⁴ This was based on a figure and description sent him by Everett Smith, of Portland, Maine, of a specimen "reported to have been taken off the Grand Banks." The length of this fish was 60 inches (1,523 mm), and the depth 25 inches (635 mm). This is apparently the record fish for size on our shores. Unfortunately its weight was not noted.

The next specimen from the western Atlantic was put on record in 1893 by Dr. R. W. Shufeldt. After noting that a specimen was said to have been taken off Sable Island many years before, he described⁵ another which he said had been caught in 1893 by Captain William T. Lee on La Have Bank in 280 feet of water. This specimen was sent to the U. S. Fish Commission in Washington, and being such a rare find it was turned over to the U. S. National Museum. A cast was made of it, colored from the data at hand, and this was exhibited at the World's Columbian Exposition in Chicago in 1893. It was the first bodily representation of this fish ever shown in any museum in the new world. This cast is now in the National Museum at Washington, but by the courtesy of that institution a replica of it hangs in the Hall of Fishes of the American Museum.

Shortly after the appearance of Shufeldt's note, Captain J. W. Collins, into whose hands the specimen in question had come immediately after its capture, made certain corrections as follows. The fish "was not caught on La Have Bank, but on the southern slope of the depressed ridge that extends eastward from that bank to the western or Sable Island Bank, and is commonly known as La Have Ridges." Furthermore, the depth was not 280 feet but 280 fathoms—1,680 feet.

Captain Collins has been quoted literally, since another specimen according to Shufeldt has come from this Sable

⁴ *Bulletin U. S. Fish Commission* for 1887, 1889, Vol. 7, p. 202.

⁵ *Forest and Stream*, 1893, Vol. 41, p. 293, fig.

Island region. Jordan and Evermann refer to this specimen in the following terms:⁶ "We have before us a drawing of *Lampris luna* made at Sable Island by James Farquhar in 1856, and sent with an accompanying letter to Dr. J. Bernard Gilpin, of Halifax." From this letter they quote as to the color of the fish, which was easily recognized.

Goode and Bean in their "Oceanic Ichthyology"⁷ refer to the specimen described by Shufeldt and give an outline drawing presumably made from the cast. They say further that "It has been reported from off Newfoundland, Nova Scotia (?) and Maine," but give no citations. On this basis, H. B. Bigelow and W. W. Welsh in their "Fishes of the Gulf of Maine"⁸ list the opah from the Gulf of Maine, but say that they have no other authority.

The one specimen known from the Gulf of Mexico was put on record by the present writer⁹ in December, 1926. This fine specimen came ashore on the bay side of Captiva Island during a heavy southwest blow early in May, 1926. It was four feet (1,218 mm) long "over all," and weighed 125 pounds. It was mounted by a local taxidermist, and this mount is now in the possession of Dr. Franklin K. Miles, of Fort Myers, Florida. This was the first and only skin to be mounted in the United States until the Boston Society's was done. As noted above, there are casts in the U. S. National and American Museums. Dr. Miles has very kindly sent me a photograph of his mounted specimen. It is very like the Boston fish, save that all the fins are shorter and broader. This may be a matter of maturity, since the Florida fish was four feet long and weighed 125 pounds, while the Massachu-

⁶ Jordan and Evermann, "Fishes of North and Middle America," Vol. I, 1896, p. 954.

⁷ Washington, 1895, p. 223.

⁸ Bulletin U. S. Bureau of Fisheries for 1924, 1925, Vol. 40 (pt. 1), p. 243.

⁹ E. W. Gudger, "The Opah or Moonfish, *Lampris luna*, on the West Coast of Florida," *Science*, 1926, Vol. 64, pp. 600-601.

setts fish was but 31.75 inches long and weighed only thirty-two pounds.

To sum the matter up, we have for our north Atlantic coast five records: one from the Grand Banks in 1887; a second from La Have Ridges in 1893; the third fish taken in 1856 at Sable Island, noted in 1893 but only definitely put on record in 1896; the fourth taken on Western Bank in 1925, and the fifth and last at Hyannis, Massachusetts, in 1928. In addition, there is a sixth fish which came ashore on Captiva Island, west coast of Florida, in 1926.

It is certainly an extraordinary thing that so few captures of the opah should have been made on our northeast coast, whereas there have been so many on the British Isles (probably at least one hundred), with scores of others reported on the coasts of western continental Europe from Portugal all the way to Scandinavia and Iceland. It would seem that *Lampris luna* is found most abundantly in the deep waters of the northeast Atlantic Ocean. However, it is found in the Pacific and for the west coast of the United States there are two or three published records and a number of unpublished ones, which I hope later to bring together in a definite faunal record.

THE NATURAL HISTORY OF THE OPAH

We know little of the natural history and habits of this rare and interesting fish, and that little pertains only to adult stages, but an effort has been made to bring this data together here in somewhat definite form. Its very paucity will at least indicate how large a field there is here for research. And it is of interest to note in passing that the best descriptions of the external form and internal anatomy of the opah are nearly one hundred years old—being found in the works of Cuvier and Valenciennes (1835) and Lowe (1843) presently to be referred to.

The opah apparently lives at considerable depths. Mr. Dimick notes that his fish was taken in deep water.

Shufeldt's specimen came from 280 fathoms (1,680 feet) according to Captain Collins. How these were taken is not stated, but most American references indicate that the fish is taken on the hook. In Madeira, where a hundred years ago it seems to have been not infrequently found in the market, Lowe¹⁰ says "This fish is taken with an ordinary bait, a whole Cavalla (Mackerel) or Chick-arro (Madeiran Horse-Mackerel), at no great distance from the shore, but at a depth of from fifty to one hundred fathoms." Still later Jenkins¹¹ notes that:

The long liners . . . say that in September every year when they are fishing to the west of the Shetlands rarely a week passes without a King-fish [*Lampris luna*] being taken, and as many as three have been obtained in one shot of the [trawl?] lines.

For all this, however, the greater number of records of the occurrence of this fish the world over have been made of specimens which have come ashore during or after storms, or have been found in shallow water near shore where they have offered comparatively little resistance to capture. The fish, as one may deduce from its shape, is not a very good swimmer.

We know next to nothing of the food of this toothless fish. Valenciennes¹² found in the stomach of one specimen the beaks of cephalopods and the remains of jelly-fishes. Lowe found in some of his specimens "small soft-coated crustacea and the remains of fish." Fulton¹³ dissected a female, 3.5 feet long, and in the stomach found "a large number of the mandibles of a cephalopod." On the basis of these three accounts and because this fish is found in deep water, we may conclude that its food consists largely of these mollusks. Just here, how-

¹⁰ R. T. Lowe, "A History of the Fishes of Madeira," London, 1843, pp. 27-35. Pl.

¹¹ J. T. Jenkins, "The Fishes of the British Isles," London, 1925, p. 77.

¹² Cuvier and Valenciennes, "Histoire Naturelle des Poissons," Paris, 1835, Vol. 10, pp. 39-60. Fig.

¹³ T. W. Fulton, "Ichthyological Notes." *Nineteenth Annual Report Fishery Board Scotland*, 1901, p. 290.

ever, Dr. Perry's observation¹⁴ from dissection of the Florida specimen is of particular interest. The stomach of this four-foot fish was full of a small clam, *Donax variabilis*. Here then we have a fish whose normal habitat is some hundreds of fathoms down, which had fed on a mollusk which, so far as is known, lives only in shallow water.

The opah is said to be a good food fish. Lowe notes that in Madeira in his day its flesh, which is "red like beef, but fine grained," was considered superior to that of the tunny, and he was told that in earlier times it was so esteemed that every fish taken there had to be carried to the governor, who issued a special license for its sale. David Starr Jordan¹⁵ says that its flesh is "of varying shades of salmon-red, tender, oily, and of a rich, exquisite flavor scarcely surpassed by any other fish whatsoever."

Little is known of the life history of the opah. Of its embryology and development we know nothing. Valenciennes dissected an unripe male whose testes were 1.5 feet long and one half to two thirds of an inch in diameter. Lowe dissected two females at Madeira. Of the first he says (without giving any date) that the ovaries were small and empty. Of the second, taken on March 30, he says, "The ovaries were two cylindrical masses, nearly as long as the stomach, and turgid with half-formed eggs." Fulton dissected a female 4.5 feet long, taken on October 18, 1901, off the Shetlands, which had well-developed ovaries. One was 290 mm (11.4 in.) long, 70 mm (2.75 in.) deep, and weighed 276 grams. The other was 240 mm (9.5 in.) long and weighed 255 grams. These organs were conical, soft, thin-walled bodies, the walls being highly vascularized. The interior of each ovary possessed a cavity, the inner surface of which was lined with an immense number of ova each in its papilla. The largest eggs measured 0.82 mm, and a slightly smaller

¹⁴ Gudger, *op. cit.*

¹⁵ "Guide to the Study of Fishes," New York, 1905, Vol. I, p. 323, fig.; Vol. II, pp. 244-245.

egg of a diameter of 0.7 mm had a germinal vesicle measuring 0.115 mm.

Judging then from these facts as to the size of the ovaries, the great number of the eggs and their very small size, one must conclude that they are pelagic. Presumably the fish extrude the eggs and sperms in deep water, whence they probably come to the surface. However, if they do this, it is certainly remarkable that, in all the vast tow-net work on plankton done in the North Atlantic, no eggs of the opah have ever been found. We have, of course, no clue to the breeding season. In this respect our lack of knowledge of the early life history of the opah parallels that of the eel, which also oviposits in deep water, but of later stages it is not true, since the larval stages of the eel—the leptocephali—are abundant and well known, while those of the opah remain absolutely unknown. Indeed, no young stages whatever have been found. At least, a search of the literature reveals no record.

The adult opah is a large and heavy fish. The smallest of which I find any record is Valenciennes' specimen of 2.5 feet long taken in the Mediterranean near Toulon. Presumably this is *measure anglaise*, which he specifically uses in the preceding paragraph. Of American and English specimens, the smallest of all is the Hyannis opah weighing thirty-two pounds. Other small specimens, taken on the coast of Great Britain, weighed fifty-one, fifty-five and fifty-nine pounds. The heaviest on record seems to be one caught near Honolulu by Mr. E. L. Berndt. Jordan¹⁶ reproduces a figure of this great fish on the scales in the fish market there. It weighed 317.5 pounds, but unfortunately no dimensions are given. The Hyannis specimen, 31.75 inches in total length, seems to be the shortest fish on record, the next above it, thirty-eight inches over all, being the Yarmouth, England, specimen weighing fifty-one pounds. How large it grows is

¹⁶ Vol. I, p. 323.

not known, but it is said to reach a length of five or six feet. Apparently the longest is one "nearly six feet" in length, captured at Sandey, Orkney Islands, as recorded by Baikie.¹⁷

The last matter to be taken up in this section of this paper is the affinities of the Lamprididae, constituted of the single genus and species, *Lampris luna*—all other alleged species being reduced to synonymy—and here we get into water as deep as that in which the fish lives. Valenciennes (1835) placed it among the other short deep-bodied Scombroids. Albert Günther¹⁸ placed it at the top of the Scombridae, next to the Carangidae. Boulenger first created the suborder Selenichthyes—moonfishes—to comprise the Lamprididae. Later, however,¹⁹ he places them next to the Gastrosteidae in the suborder Catostomei.

Now the final settlement of the relationship and place in the ichthyological system of any fish is by common agreement based chiefly on the bony structures, since these, however much they may be modified, are always found in the same relative forms and positions. To give credit where credit is due it should be stated that the two earliest researchers on the osteology of the opah were Gerhard Bakker,²⁰ whose drawings are to this day unsurpassed by those of any other student, and Emil Ekhoﬀ, whose paper was offered as an academic dissertation at the University of Upsala and published separately at Stockholm in 1875.

However, to ascertain the place of *Lampris* it has been necessary to study the skeletons, and particularly the skulls of the related forms. This has been done by C. T.

¹⁷ W. B. Baikie, "Catalogue of the Fishes of Orkney and Zetland," *Zoologist*, 1853, Vol. 11, p. 3951.

¹⁸ "Catalogue of Acanthopterygian Fishes in the British Museum," 1860, Vol. II, p. 416.

¹⁹ "Fishes" (Systematic Account of Teleostei), in "Cambridge Natural History," London, 1904, Vol. VII, pp. 626-623.

²⁰ Gerhard Bakker, "Osteographia Piscium," Groningae, 1822, text and plates.

Regan,²¹ of the British Museum, who has studied the skulls of the short deep-bodied fishes *Lampris* and *Velifer*, the greatly elongated dealfish (*Trachypterus*), oarfish (*Regalecus*) and the crested bandfish (*Lophotes*). As a result of this research, Regan has found a marked similarity of the high arched skull in general appearance and in minute details. As a result of this work he has established the suborder Allotriognathi to include the highly specialized fishes of the families Lamprididae (with one genus), Veliferidae (also with one genus), Trachypteridae (two genera) and Lophotidae (two genera). He finds further that the Allotriognathi and the Beryciformes are remarkably close to each other. The reader interested in the skull structures and the affinities of the opah and these other fishes grouped in this new suborder will find the whole matter set out in Regan's paper above referred to. The present-day tendency among systematic ichthyologists is to accept this arrangement as a sound one.

²¹ C. T. Regan, "On the Anatomy, Classification and Systematic Position of the Teleostean Fishes of the Suborder Allotriognathi," *Proceedings Zoological Society London*, 1902, pt. 2, pp. 634-643. 6 text-figs.

SHORTER ARTICLES AND DISCUSSION
A NOTE ON THE SIDEWINDING LOCOMOTION OF
SNAKES¹

THE peculiar type of ophidian locomotion known as sidewinding has been noted and described by a number of authors, for instance, Hutchinson (1879), Wall (1907; 1926), Ditmars, and perhaps by none better than by L. M. Klauber (1927). This author states that:

The sidewise, looping motion [of *Crotalus cerastes*], which gives it its name, is accentuated when the snake is excited or in a hurry. At such times the middle section of the body is thrown out in a loop. This section is then anchored and the balance of the body is drawn laterally in a line perpendicular to the direction in which the snake is facing. The action is so continuous that the snake has the appearance of flowing sidewise over the ground. The snake may move with equal facility either to the right or left.

When proceeding somewhat more slowly the snake draws the central portion of the body forward in a sharp loop, the apex of the loop being well in advance of the head. The head is then advanced and the snake thus proceeds in the direction in which it is facing rather than laterally.

A similar account was written by the present writer as a result of his experience with *Cerastes vipera* in the Sahara in 1926. Two years later the opportunity was presented for studying the sand tracks of several Sahara reptiles. In order to explain the curious tracks of *Cerastes*, the writer observed this snake carefully in captivity. Many instantaneous photographs taken during movement aided in the correct analysis of the latter. Even then the writer did not recognize the mechanical principle of the movement and the significance for the knowledge of snake movement in general.

In order to make clear the principle of this movement a diagram has been prepared to show the relative positions of a portion of the snake's body during movement (Fig. 1). It will be noted that this segment is in the form of a double curve, of which the cranial convexity is directed backwards and the caudal convexity is directed forwards. Between the two convexities there is found a more or less straight section of the body (x), the caudal part of which lies obliquely forward while the cranial end

¹ From the departments of anatomy and zoology and the Museum of Zoology, University of Michigan.

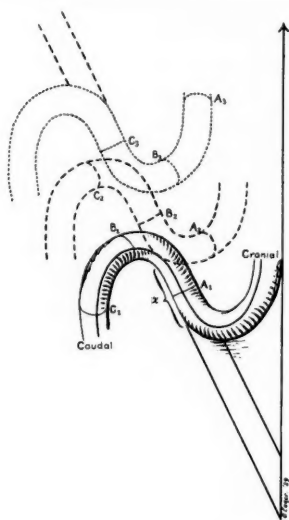


FIG. 1. One S-shaped segment of *Cerastes* during locomotion, showing three successive phases. A_1 , A_2 and A_3 indicate the position of one and the same point of the snake's body as seen in certain intervals. The same applies to B_1 , B_2 , B_3 and C_1 , C_2 , C_3 . The arrow gives the direction of the course; x is the segment in contact with the ground.

is directed obliquely backward. Only this straight part of the double curve is in contact with the ground. The curves themselves are distinctly lifted, and their vertices are lifted highest. In movement this whole S-shaped piece rolls like a wheel on a straight track, the direction of which is indicated by the position of the straight part x , the only part in contact with the ground. The curves flow caudalward along the body. That the movement is essentially a rolling movement may be seen from the fact that successive points, as for instance A , B and C (Fig. 1), form in regular sequence the vertex of the caudal curve, are then set down, touch the sand firmly for a moment, serving as a fulcrum, and are then lifted again to form the cranial curve. Thus the point of contact with the ground ("point d'appui") migrates posteriorly on the snake, finally reaching the tip of the tail.

As in the rolling of a wheel there is no translation or sliding of the single points on the substratum. Each point of the body is set down on a certain point of the sand, remains there a moment and is lifted again. Naturally only the parts just touching the sand leave impressions in it, so that the track is formed

by fulcrum x. Since each point of the body in succession is used as the fulcrum, the length of one track must be exactly that of the snake itself.

The track picture resembles a ladder, the rounds of which are formed by the sharp impressions of the single ventral scutes in the sand. This fact is the proof that there is no gliding movement, for otherwise there would be no sharp impressions.

The movement so far described is only half that which actually takes place during the locomotion of the snake. That is to say, there are simultaneously two double curves with two points of contact with the ground (Fig. 2). Locomotion thus consists of



FIG. 2. Diagram of *Cerastes* in locomotion. The tracks already left are indicated by the unbroken lines; the future parts of them by the dotted lines. The arrow gives the direction of the course; x indicates the two segments in contact with the ground.

two rolling motions running parallel to each other and resulting in the formation of two tracks at once. An exactly similar track is left by the rolling of a circular helix of slightly less than two turns. If a helix is made of soft wire about one hundred centimeters long, the diameter of the turns being about fifteen centimeters and the distance between them ten or twelve centimeters, it will roll along leaving on a sanded floor a series of straight tracks, each as long as the wire (one hundred centimeters),

parallel, ten or twelve centimeters apart, and inclined at about 16° to the direction of motion. Such parallel, disconnected and equidistant tracks, each as wide as the snake's body, are actually left by a sidewinding snake in soft sand, or by a wet snake on a smooth floor. It is obvious of course that the "snake's body" in the wire helix is ventral side up on top of the loops, which constitutes an essential difference between the rigid wire model and the living and flexible animal. But the tracks left by the two are so much alike that the model is helpful and enlightening. The rolling helix in fact better represents the snake's progress than any other mechanical model so far described.

As such a spiral rolls, there is an instant when the rear end (the "tail") lifts from the ground. The spiral has now only one turn in contact with the ground. A corresponding phase occurs in the snake's movement. After the tail has formed the anterior vertex of the caudal curve and then the fulcrum x , its tip is dragged slightly forward, forming a hook in the track, before being raised from the ground. For a moment now the snake is supported only by one part in contact with the ground. Almost immediately a new point of contact with the ground is made by the neck, which, before it is set down, is thrown into a sharp curve, the beginning of a new S-shaped segment, and a new track thus is begun in advance of the old, which is continued in the meantime by the old contact x , rolling farther toward the tail.

The type of movement described seems to be caused by the lack of resistance in the sandy substratum. The writer concluded that other snakes ought to show a similar movement under similar conditions, on a smooth surface, and such has proved to be the case. That sidewinding is not restricted to the snakes in which it has been recognized is indicated by the behavior of such snakes as *Thamnophis sirtalis*, the garter snake, and *Storeria dekayi*, the brown snake. These snakes are capable of adopting a movement essentially like the sidewinding of *Cerastes vipera* when they are obliged to move on a smooth floor.

It is of particular interest that there is in this type of locomotion a certain amount of vertical movement represented by the lifting of the curves, a fact which has been strictly denied by most authors who have discussed locomotion in snakes. This vertical movement plays an important rôle in progression. Furthermore, there is no gliding movement.

The writer has recently had the opportunity of observing the sidewinder (*Crotalus cerastes*).² Locomotion in this snake is of the same type as in *Cerastes vipera*. The tracks formed are also alike. However, there are a few unimportant differences, due apparently to the difference in the shape of the body. Since *Crotalus* is relatively longer, it seems not to need its full length, and therefore the cranial curve is formed a few centimeters behind the head instead of on its foremost portion, as in *Cerastes*.

It is proposed to investigate the locomotor movements of snakes in general and the anatomical basis for limbless progression.

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SALINITY AND THE SIZE OF *UROSALPINX CINEREA* SAY

THE relationship existing between the size of the individuals of a marine form and the salinity at which the animals have been reared has been reported upon in some few cases, but only in a general way (Vernon, 1903; Huntsman, 1919; Flattely and Walton, 1922; Hubbs, 1926). From these observations two opposed conclusions are to be derived. The first states that, within certain limits, size is directly proportional to the salinity (Hubbs, 1926); the other maintains that the more saline waters tend to restrict the size attained (Vernon, 1903; Flattely and Walton, 1922).

² Through the courtesy of the Museum of Zoology, University of Michigan.

The former statement refers in general to the influence of salinity on the size of fishes; the latter refers to some exceptions among fishes, to mollusks, and to echinoderm larvae.

In the following note there are given some observations on the relation between salinity and the size of *Urosalpinx cinerea*, with a short discussion of the interpretation of these data.

During the course of some studies on the natural history of *Urosalpinx cinerea* Say, data were collected on the average size of this species from two localities: Norfolk, Virginia, and Beaufort, North Carolina. The former were collected during 1927; the latter during 1928. Size was taken as the length from the apex of the shell to the tip of the siphon. Several hundred individuals from both places, collected in similar ways and selected at random, were measured. These figures are plotted as size groups versus frequency, in Fig. 1.

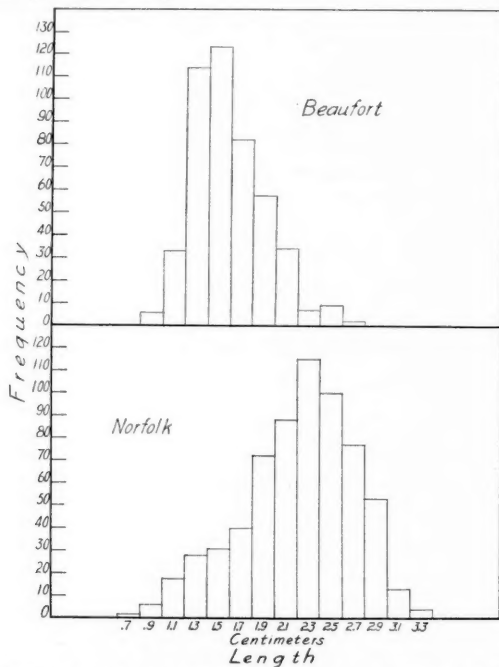


FIG. 1. Curves showing the frequency of the size groups of *Urosalpinx cinerea* from Beaufort and from Norfolk. Size is taken as the length from the apex of the shell to the tip of the siphon.

It is apparent that the average size of *Urosalpinx cinerea* is different in the two localities. At Norfolk the average length is between 21 and 25 millimeters; the animals from Beaufort, on the other hand, average approximately 13 to 17 millimeters. That this difference in size is not due altogether to temperature is shown by Fig. 2, in which are plotted weekly temperatures for

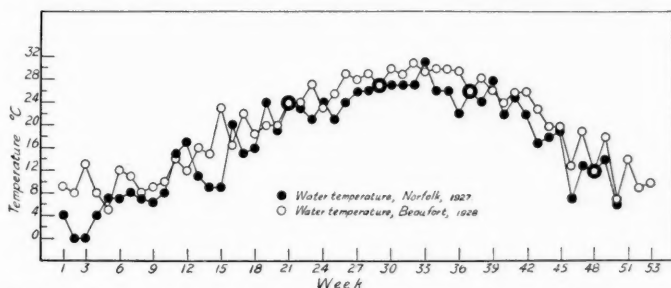


FIG. 2. Curve giving water temperatures at weekly intervals at Beaufort and at Norfolk.

the two regions. The temperature difference between the two regions is small, rarely exceeding 4° C., which although significant to some degree seems not to be of sufficient magnitude to cause such a great difference in length. On the other hand, the salinities for the two regions differ greatly (Fig. 3), there being an average salinity difference of approximately ten parts per mille. The salinities for Norfolk were taken at Craney Island, while the

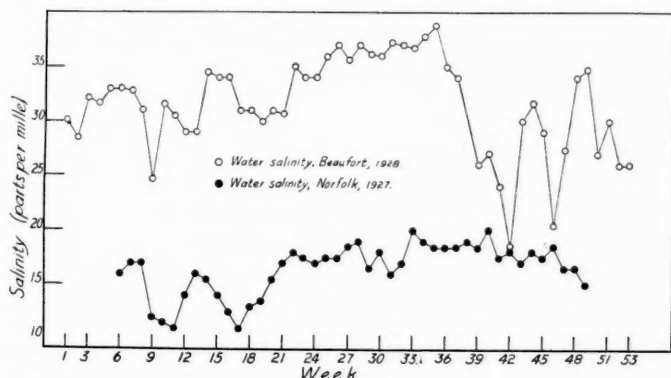


FIG. 3. Curve showing salinities at weekly intervals at Beaufort and at Norfolk. The salinities were calculated from specific gravity readings.

animals were collected at various places in Hampton Roads. Comparison of salinities in the two localities at various times throughout the year showed that at Hampton Roads the salinity averaged about three to five parts per mille higher than that at Craney Island. Thus we must deduct this from the difference in the salinity between Beaufort and Norfolk as shown in Fig. 3.

Since these are observational data the writer does not wish to maintain that the difference in size is due solely, or even partly, to salinity. Other factors, such as the amount of sunlight which indirectly determines the vitamin content (*i.e.*, the number and kind of algae) of the sea water, or some difference in the chemical constituents of the two waters, may be the controlling factors. These results do, however, corroborate Vernon's (1903) experimental work on the sea-urchin *plutei*. He states, "These *plutei*, allowed to develop in sea water of various concentrations, attained a greater size than the normal when kept in moderately diluted water, and probably a slightly smaller size when kept in concentrated water." In 1922 Flattely and Walton wrote, "In many estuaries . . . mussels may be distributed from the lowest outlet to a point a couple of miles upstream. . . . Only those mussels, however, which are situated some distance up from the mouth of the estuary are capable of growing to marketable size," indicating that mussels grow larger in brackish water. Furthermore, it is a well-known fact that oysters attain a larger size in brackish than in saline waters. Hubbs (1926), whose data are obtained from the works of other investigators, states: "The abrupt and extensive retardation of growth under accelerating conditions of development explains the *general observation that fish of cold or saline waters usually attain a larger size than do individuals or races of the same species inhabiting warm or brackish waters or both.*"¹

The contradictory observations on mollusks and echinoderms, on the one hand, and fishes on the other, may have some explanation in the developmental history of these animals. Fishes usually travel great distances during their development, so that fishes collected in brackish waters may have had a larval stage in the open waters of the sea. The converse may also be true. Unless we know, therefore, the salinities of the waters in which the animals have developed, it seems to the author difficult to find any correlation between salinity and size. In *Urosalpinx cinerea* the

¹ Italics mine.

eggs are laid within a capsule attached to the substrate. Development takes place there and the animal hatches as a small snail, fully developed, excepting as to size. The animal does not migrate very extensively (unpublished observations) and unless it is moved by oystermen during their oyster-planting activities the snail remains limited to a definite area. This is not so true of oysters and mussels but even these animals do not show such wide-spread migrations as fishes. It may very well be, therefore, that the contradictory data obtained among the fishes may eventually be explained when we know more about the life-histories of these species (Huntsman, 1919; Hubbs, 1926). On the other hand, we may find that the various species have different optimum salinities (Vernon, 1903), and that lower or higher salinities produce smaller sizes. These smaller animals might be called *sternomorphs* if we extend the original meaning of the term (Bartsch, 1923) to include not only the diminutive forms produced by a cramped habitat, but all dwarfed individuals which are produced by changes in other environmental factors such as, for instance, salinity.²

SUMMARY

Urosalpinx cinerea Say were collected from two localities (Beaufort, North Carolina, and Norfolk, Virginia), having different salinities but only slightly dissimilar temperatures and their lengths measured. The results indicate that the animals grow to larger size in brackish than in saline waters. An attempt is made to explain the contradictory results in other forms.

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² Since writing this, Mr. J. H. Fraser, of the University of Liverpool, advises me that *Urosalpinx cinerea* Say collected from the River Blackwater oyster beds, where the means of the maximum and the minimum salinities per mille were respectively 34.0 and 34.53 in 171 observations, from June, 1921, to October, 1924, averaged 29-30 mm in length. A possible explanation for this large size may be found in the fact that *U. cinerea* Say has only recently been discovered in England (Orton and Winckworth, 1928) and has therefore been subjected to a new set of environmental conditions.

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GRAYED HAIR AS AN INDEX OF RELATIVE EFFECTIVE X-RAY DOSAGE IN THE HOUSE MOUSE

In experiments involving the temporary sterilization of mammals by X-rays, it is advisable to have an index of effective dosage because the cone of rays from a therapeutic machine varies in intensity from point to point on the same cross-sectional plane, due to irregularities at the source. Hence mice treated at the same time under theoretically identical conditions may vary greatly in their physiological response to the ray treatment.

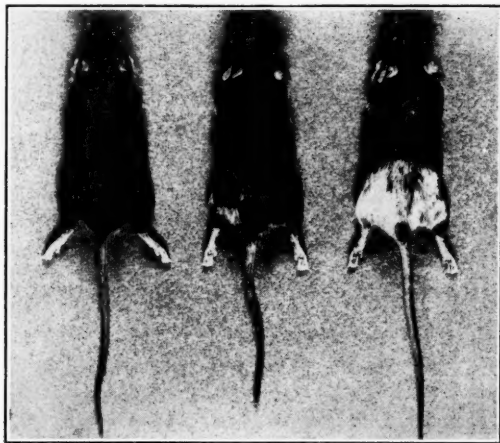


FIG. 1

1
Fertile
4 days
Sterile
39 days

2
Sterile
at once
Sterile
47 days

3
Sterile
at once
Sterile
61 days

Three black male mice (Fig. 1) were mechanically immobilized and treated in such a fashion that their hind quarters only were exposed to the cone of rays directed vertically upward from beneath. The remainder of the body of each animal was protected by a lead shield.

Breeding tests were subsequently made with these animals. The first animal (Fig. 1) contained fertile sperm four days after the treatment, and then went into a period of sterility lasting thirty-nine days.

The second animal became sterile at once and remained so for forty-seven days.

The third became sterile at once and continued so for sixty-one days.

Graying in the coat of the mouse subsequent to X-ray treatment has been described in detail by Hance (1927).

It will be noted that the amount of graying of the pelage (Fig. 1) exposed to the rays is directly correlated with the length of time that the animal remained sterile. Thus, the amount of graying may be used as an index of the relative physiological effectiveness of the treatment upon different individuals.

It is interesting to note that the cone of rays spreads forward through the body at an angle of about 31 degrees (Fig. 2) and affects the dorsal hair much farther anterior than its limit on the ventral side. This is probably due to the scattering of secondary

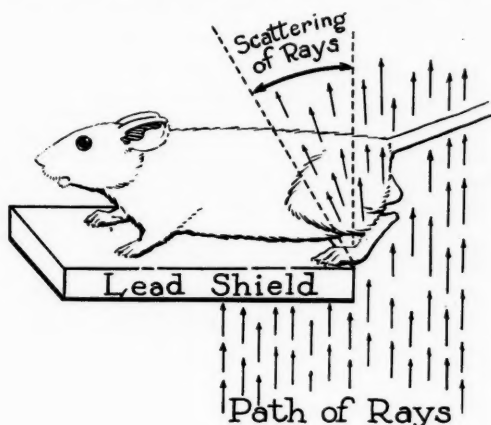


FIG. 2

Diagram showing the scattering of secondary rays as detected by the grayed areas upon the experimental mice.

rays produced by the incidence of primary rays with the tissues of the animal.

Mouse No. 1 is a heterozygote for albinism, while Nos. 2 and 3 are not, showing that heterozygosity for albinism is not a factor involved in the whitening.

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A FERTILE TETRAPLOID HYBRID BETWEEN *EUCHLAENA PERENNIS* AND *ZEA MAYS*¹

IN connection with genetic studies of the meiotic distribution of chromosomes in perennial teosinte-maize hybrids (Emerson, 1929), a plant has been observed among the F_1 individuals that differs markedly from its sibs and resembles in many ways the F_1 hybrids between annual teosinte and maize. The possibility that the maize parent had contributed two sets of chromosomes or that one maize set had doubled after fertilization immediately suggested itself. An examination of root tips from this plant showed that there are forty chromosomes present, indicating that the suggested explanation is probably correct.

It has long been known that annual teosinte and maize hybridize readily to produce fertile progenies. Longley (1924) and Randolph (unpublished) have found that the reduced number of chromosomes in annual teosinte is the same as that of maize, namely, ten. Both these investigators have found, as might have been predicted from the fact of the fertility of the hybrid, that chromosome complements from the two species show synaptic compatibility, going through the meiotic divisions in the hybrids in a regular manner.

Longley (1924) and Randolph (unpublished) have found that perennial teosinte has forty chromosomes as the unreduced number. Synapsis is in pairs according to Longley, and most frequently in groups of four (quadrivalents) according to Randolph. Both agree that, in the hybrids which are readily made with maize, there are thirty chromosomes that tend strongly to

¹ Paper No. 170, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

synapse by twos, with the third homologue loosely attached to form a trivalent or unattached as a univalent. Because of the triploid nature of this hybrid, a large proportion of the spores fail to give rise to functional gametes. The sterility of this hybrid can, at least largely, be attributed to its triploid nature, though with the information now at hand one can not say that the chromosomes of *Euchlaena perennis* are entirely compatible with those of *Zea mays*, as appears to be the case with the chromosomes of *Euchlaena mexicana*.

F₁ hybrids between perennial teosinte and maize are much more teosinte-like than are hybrids between annual teosinte and maize as described in detail by Collins and Kempton (1920). They are weakly perennial with few or no rhizomes, show a response to length of day similar to that of teosinte (Emerson, 1924) and in morphology resemble teosinte much more strongly than do the annual teosinte-maize hybrids. That the differences between the F₁ hybrids of the two species of teosinte with maize are largely the result of the difference in ratio of parental chromosomes is made evident by the occurrence of a hybrid between perennial teosinte and maize in which twenty chromosomes (two sets) are derived from each parent. This hybrid, with an equal contribution from the two parents, resembles the normal hybrid between annual teosinte and maize in which there is likewise an equal contribution from the two parents. This tetraploid hybrid differs from the annual teosinte-maize hybrid in being weakly perennial, a tendency presumably contributed by the perennial teosinte parent. The perennial tendency, as might be expected, is less strongly developed than in the normal triploid hybrid of *Euchlaena perennis* and *Zea mays*.

Female inflorescences of an annual form of teosinte, of the perennial species and of their hybrids with maize are shown in Fig. 1. The similarity between the annual teosinte-maize hybrid and the perennial teosinte-maize hybrid with forty chromosomes is apparent. Both hybrids have "four-rowed" spikes. The contrast between these and the "two-rowed" inflorescence of the perennial teosinte-maize hybrid with thirty chromosomes is obvious.

Like the annual teosinte-maize hybrid, the tetraploid hybrid is fertile. Fertility in such a hybrid is to be expected. With a 2-n contribution from each parent, fertility would be expected even in interspecific hybrids in which the chromosome of one species showed complete failure of synapsis with those of the

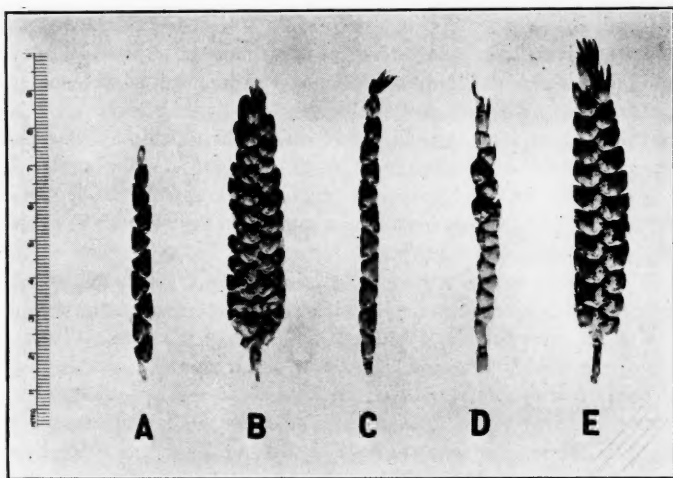


FIG. 1. Pistillate spikes of annual and perennial species of teosinte and their hybrids with maize. *A.* Chalco teosinte (annual). *B.* F_1 hybrid between Chalco teosinte and maize. *C.* Perennial teosinte. *D.* A normal thirty-chromosome hybrid between perennial teosinte and maize. Note that only two "seeds" have developed. *E.* A forty-chromosome hybrid between perennial teosinte and maize. Each parent has contributed twenty chromosomes.

other (Renner, 1929). Meiosis in the tetraploid hybrid under discussion has not as yet been studied, so that the type of synapsis is not known.

The occurrence of such a hybrid as described above offers possibilities for several interesting genetical and cytological studies.

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